

Baskar  
091868292

09/868293

(FILE 'REGISTRY' ENTERED AT 14:30:11 ON 30 OCT 2003)  
L1 14 S TTESLETLV/SQSP

Seg. ID 2

L1 ANSWER 1 OF 14 REGISTRY COPYRIGHT 2003 ACS on STN  
RN 556707-17-6 REGISTRY  
CN GenBank AAP98013 (9CI) (CA INDEX NAME)  
OTHER NAMES:  
CN GenBank AAP98013 (Translated from: GenBank AE017157)  
CI MAN  
SQL 129

SEQ 1 MTTESLETLV EKLSNLTVLE LSQKKLLEE KWDVTASAPV VAVAAGGGGE  
===== =  
51 APVAAEPTEF AVTLEDVPAD KKIGVLVVR EVTGLALKEA KEMTEGLPKT  
101 VKEKTSKSDA EDTVKKLQDA GAKASFKGL

HITS AT: 2-11

\*\*RELATED SEQUENCES AVAILABLE WITH SEQLINK\*\*

L1 ANSWER 2 OF 14 REGISTRY COPYRIGHT 2003 ACS on STN  
RN 477027-28-4 REGISTRY  
CN Protein (Chlamydia trachomatis clone CTR200582 essential) (9CI) (CA INDEX NAME)  
OTHER NAMES:  
CN 1215: PN: WO02077183 SEQID: 55215 claimed protein  
CI MAN  
SQL 130

SEQ 1 MTTESLETLV EQLSGLTGLE LSQKKMLEE KWDVTAAAPV VAVAGAAAAG  
===== =  
51 DAPASAEPTF FAVILEDVPA DKKIGVLKVV REVTLGLALKE AKEMTEGLPK  
101 TVKEKTSKSD AEDTVKKLQE AGAKAVAKGL

HITS AT: 2-11

\*\*RELATED SEQUENCES AVAILABLE WITH SEQLINK\*\*

REFERENCE 1: 138:1094

L1 ANSWER 3 OF 14 REGISTRY COPYRIGHT 2003 ACS on STN  
RN 477023-92-0 REGISTRY  
CN Protein (Chlamydia pneumoniae clone CPN200683 essential) (9CI) (CA INDEX NAME)  
OTHER NAMES:  
CN 876: PN: WO02077183 SEQID: 54876 claimed protein  
CI MAN  
SQL 129

SEQ 1 MTTESLETLV EKLSNLTVLE LSQKKLLEE KWDVTASAPV VAVAAGGGGE  
===== =  
51 APVAAEPTEF AVTLEDVPAD KKIGVLVVR EVTGLALKEA KEMTEGLPKT  
101 VKEKTSKSDA EDTVKKLQDA GAKASFKGL

HITS AT: 2-11

\*\*RELATED SEQUENCES AVAILABLE WITH SEQLINK\*\*

REFERENCE 1: 138:1094

L1 ANSWER 4 OF 14 REGISTRY COPYRIGHT 2003 ACS on STN

Searcher : Shears 308-4994

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RN 462351-19-5 REGISTRY  
CN GenBank BAA98290 (9CI) (CA INDEX NAME)  
OTHER NAMES:  
CN GenBank BAA98290 (Translated from: GenBank AP002545)  
CI MAN  
SQL 129

SEQ 1 MTTESLETLV EKLSNLTVLE LSQLKKLLEE KWDVTASAPV VAVAAGGGGE  
===== =  
51 APVAAEPTEF AVTLEDVPAD KKIGVLKVVR EVTGLALKEA KEMTEGLPKT  
101 VKEKTSKSDA EDTVKKLQDA GAKASFKGL

HITS AT: 2-11

\*\*RELATED SEQUENCES AVAILABLE WITH SEQLINK\*\*

FILE 'HCAPLUS' ENTERED AT 14:31:10 ON 30 OCT 2003  
L2 10 S L1

L2 ANSWER 1 OF 10 HCAPLUS COPYRIGHT 2003 ACS on STN  
ACCESSION NUMBER: 2002:781490 HCAPLUS  
DOCUMENT NUMBER: 138:1094  
TITLE: Essential genes in microorganisms and their use  
as targets for antisense inhibition of  
proliferation and antibiotic screening  
INVENTOR(S): Wang, Liangus; Zamudio, Carlos; Malone, Cheryl;  
Haselbeck, Robert; Ohlsen, Kari L.; Zyskind,  
Judith W.; Wall, Daniel; Trawick, John D.; Carr,  
Grant J.; Yamamoto, Robert; Forsyth, R. Allyn;  
Xu, H. Howard  
PATENT ASSIGNEE(S): Elitra Pharmaceuticals, Inc., USA  
SOURCE: PCT Int. Appl., 1766 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 22  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002077183	A2	20021003	WO 2002-XM9107	20020321
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DE, DK, DK, DM, DZ, EC, EE, EE, ES, FI, FI, GB, GD, GE, GH, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG			
RW:	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG			
US 2002061569	A1	20020523	US 2001-815242	20010321
WO 2002077183	A2	20021003	WO 2002-US9107	20020321
W:	AE, AG, AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, CZ, DE, DE, DK, DK, DM, DZ, EC, EE, EE, ES, FI, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD,			

Searcher : Shears 308-4994

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SE, SG, SI, SK, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US,  
UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG  
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE,  
CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT,  
SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE,  
SN, TD, TG

PRIORITY APPLN. INFO.:

US 2001-815242 A 20010321  
US 2001-948993 A 20010906  
US 2001-342923P P 20011025  
US 2002-72851 A 20020208  
US 2002-362699P P 20020306  
WO 2002-US9107 A 20020321  
US 2000-191078P P 20000321  
US 2000-206848P P 20000523  
US 2000-207727P P 20000526  
US 2000-242578P P 20001023  
US 2000-253625P P 20001127  
US 2000-257931P P 20001222  
US 2001-269308P P 20010216

AB The sequences of antisense nucleic acids which inhibit the proliferation of prokaryotes are disclosed. Thus, 6213 nucleic acid fragments are identified for which expression inhibits proliferation or is required for proliferation in Enterococcus faecalis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, and Staphylococcus aureus. Cell-based assays which employ the antisense nucleic acids to identify and develop antibiotics are also disclosed. The antisense nucleic acids can also be used to identify proteins required for proliferation, express these proteins or portions thereof, obtain antibodies capable of specifically binding to the expressed proteins, and to use those expressed proteins as a screen to isolate candidate mols. for rational drug discovery programs. The nucleic acids can also be used to screen for homologous nucleic acids that are required for proliferation in cells other than Staphylococcus aureus, Salmonella typhimurium, Klebsiella pneumoniae, and Pseudomonas aeruginosa. The invention provides 38,184 such proliferation-required gene sequences (plus their encoded protein sequences). The nucleic acids of the present invention can also be used in various assay systems to screen for proliferation required genes in other organisms. [This abstract record is one of twenty records for this document necessitated by the large number of index entries required to fully index the document and publication system constraints.].

IT 477023-92-0 477027-28-4

RL: BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses)  
(amino acid sequence; essential genes in microorganisms and their use as targets for antisense inhibition of proliferation and antibiotic screening)

L2 ANSWER 2 OF 10 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER:

2001:753815 HCAPLUS

DOCUMENT NUMBER:

135:287534

TITLE:

Antibody for immunoassay of Chlamydia trachomatis

INVENTOR(S):

Monzul, Larman; Eto, Takashi

PATENT ASSIGNEE(S):

Asahi Chemical Industry Co., Ltd., Japan

SOURCE:

Jpn. Kokai Tokkyo Koho, 13 pp.

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CODEN: JKXXAF

DOCUMENT TYPE:

Patent

LANGUAGE:

Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2001286295	A2	20011016	JP 2001-24749	20010131
PRIORITY APPLN. INFO.:			JP 2000-62685	A 20000131
AB Monoclonal antibodies and polyclonal antibodies to ribosomal protein L7/L12 were prepared by hybridoma and known methods. These antibodies are highly specific to the C. trachomatis ribosomal protein, and are useful for high-accuracy immunoassay of the C. trachomatis. Cloning of gene for the ribosomal protein L7/L12 of C. trachomatis, recombinant manufacture of the protein with Escherichia coli, and preparation of monoclonal and polyclonal antibodies were shown.				
IT	215102-41-3			
RL: PRP (Properties); THU (Therapeutic use); BIOL (Biological study); USES (Uses) (amino acid sequence; antibody for immunoassay of Chlamydia trachomatis)				

L2 ANSWER 3 OF 10 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 2001:581938 HCAPLUS  
DOCUMENT NUMBER: 135:166019  
TITLE: Antibody for detecting Chlamydia pneumoniae  
INVENTOR(S): Rahman, Monzur; Etoh, Takashi  
PATENT ASSIGNEE(S): Asahi Kasei Kabushiki Kaisha, Japan  
SOURCE: PCT Int. Appl., 30 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: Japanese  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001057089	A1	20010809	WO 2001-JP625	20010131
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			
PRIORITY APPLN. INFO.:			JP 2000-62684	A 20000131
AB A method for specifically, highly sensitively and quickly detecting a microorganism belonging to Chlamydia pneumoniae; an antibody to be used in the detection; a detection reagent kit; and a process for producing the antibody to be used in the detection. Namely, an antibody against the ribosomal protein of a microorganism belonging to C. pneumoniae which reacts specifically with this microorganism;				

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a method of detecting the microorganism in a specimen by using this antibody; and a detection reagent kit containing this antibody. The ribosomal protein is exemplified by ribosomal protein L7/L12 and this method is usable in detecting the infection with a microorganism causative of pneumonia.

IT 353850-95-0

RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)  
(amino acid sequence; anti-ribosomal protein antibodies for detecting Chlamydia pneumoniae)

IT 215102-41-3

RL: PRP (Properties)  
(unclaimed sequence; antibody for detecting Chlamydia pneumoniae)

REFERENCE COUNT: 13 THERE ARE 13 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 4 OF 10 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 2000:511103 HCAPLUS

DOCUMENT NUMBER: 134:232463

TITLE: Comparison of outer membrane protein genes omp and pmp in the whole genome sequences of Chlamydia pneumoniae isolates from Japan and the United States

AUTHOR(S): Shirai, Mutsunori; Hirakawa, Hideki; Ouchi, Kazunobu; Tabuchi, Mitsuaki; Kishi, Fumio; Kimoto, Mitsuaki; Takeuchi, Hiroaki; Nishida, Junko; Shibata, Kaori; Fujinaga, Ryutaro; Yoneda, Hiroshi; Matsushima, Hiroshi; Tanaka, Chiho; Furukawa, Susumu; Miura, Koshiro; Nakazawa, Atsushi; Ishii, Kazuo; Shiba, Tadayoshi; Hattori, Masahira; Kuhara, Satoru; Nakazawa, Teruko

CORPORATE SOURCE: Departments of Microbiology, Kanagawa, Japan

SOURCE: Journal of Infectious Diseases (2000),  
181(Suppl. 3), S524-S527

CODEN: JIDIAQ; ISSN: 0022-1899

PUBLISHER: University of Chicago Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Chlamydia pneumoniae is a widespread pathogen of the respiratory tract that is also associated with atherosclerosis. The whole genome sequence was determined for a Japanese isolate, C. pneumoniae strain J138. The sequence predicted a variety of genes encoding outer membrane proteins (OMPs) including ompA and porB, another 10 predicted omp genes, and 27 pmp genes. All were detected in the whole genome sequence of strain CWL029, a strain isolated and sequenced in the United States. A comparative study of the OMPs of the two strains revealed a nucleotide sequence identity of 89.6-100% (deduced amino acid sequence identity, 71.1-100%). The overall genomic organization and location of genes are identical in both strains. Thus, a few unique sequences of the OMPs may be essential for specific attributes that define the differential biol. of two C. pneumoniae strains.

IT 223709-50-0

RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)  
(amino acid sequence; comparison of outer membrane protein genes

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omp and pmp in the whole genome sequences of Chlamydia pneumoniae isolates from Japan and the United States)

REFERENCE COUNT: 20 THERE ARE 20 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 5 OF 10 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 2000:441819 HCAPLUS  
DOCUMENT NUMBER: 133:72938  
TITLE: Chlamydia trachomatis antigens  
INVENTOR(S): Ratti, Giulio  
PATENT ASSIGNEE(S): Chiron S.p.A., Italy  
SOURCE: PCT Int. Appl., 25 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000037494	A2	20000629	WO 1999-IB2065	19991217
WO 2000037494	A3	20001012		
W: CA, JP, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
CA 2355876	AA	20000629	CA 1999-2355876	19991217
EP 1140997	A2	20011010	EP 1999-958455	19991217
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2002534062	T2	20021015	JP 2000-589563	19991217
PRIORITY APPLN. INFO.:			GB 1998-28000	A 19981218
			WO 1999-IB2065	W 19991217

AB Proteins encoded by Chlamydia trachomatis which are immunogenic in humans as a consequence of infection have been identified using Western blots of two-dimensional electrophoretic maps. Several known immunogens were identified, as were proteins not previously known to be immunogens, and proteins not previously reported as expressed gene products.

IT 278807-55-9

RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)  
(Chlamydia trachomatis antigens)

L2 ANSWER 6 OF 10 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 2000:335519 HCAPLUS  
DOCUMENT NUMBER: 133:1493  
TITLE: Chlamydia pneumoniae genome sequence  
INVENTOR(S): Stephens, Richard; Mitchell, Wayne; Kalman, Sue; Davis, Ronald  
PATENT ASSIGNEE(S): The Regents of the University of California, USA  
SOURCE: PCT Int. Appl., 330 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

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PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000027994	A2	20000518	WO 1999-US26923	19991112
WO 2000027994	A3	20001123		
W:	AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG			
AU 2000017223	A5	20000529	AU 2000-17223	19991112
EP 1133572	A2	20010919	EP 1999-960323	19991112
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO			
JP 2002529069	T2	20020910	JP 2000-581161	19991112
PRIORITY APPLN. INFO.:			US 1998-108279P	P 19981112
			US 1999-128606P	P 19990408
			WO 1999-US26923	W 19991112

AB The *Chlamydia pneumoniae* genome sequence and anal. of the encoded polypeptides and RNAs are provided. The *C. pneumoniae* genome contains 187,711 addnl. nucleotides compared to the *C. trachomatis* genome, and the 214 coding sequences not found in *C. trachomatis* account for most of the increased genome size. The majority of these addnl. genes lack identifiable homologs to genes from other organisms, and probably are essential for specific attributes that define the differential biol., tropism, and pathogenesis of *C. trachomatis* and *C. pneumoniae*. The *C. pneumoniae* gene nucleic acid compns. find use in identifying homologous or related proteins and the DNA sequences encoding such proteins; in producing compns. that modulate the expression or function of the protein; and in studying associated physiol. pathways. In addition, modulation of the gene activity *in vivo* is used for prophylactic and therapeutic purposes, such as identification of cell type based on expression, and the like.

IT 271232-35-0P

RL: ANT (Analyte); BPN (Biosynthetic preparation); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); PREP (Preparation); USES (Uses)  
(amino acid sequence; *Chlamydia pneumoniae* genome sequence)

L2 ANSWER 7 OF 10 HCPLUS COPYRIGHT 2003 ACS on STN  
ACCESSION NUMBER: 2000:225326 HCPLUS  
DOCUMENT NUMBER: 132:246932  
TITLE: Genome sequences of *Chlamydia trachomatis* MoPn and *Chlamydia pneumoniae* AR39  
AUTHOR(S): Read, T. D.; Brunham, R. C.; Shen, C.; Gill, S. R.; Heidelberg, J. F.; White, O.; Hickey, E. K.; Peterson, J.; Utterback, T.; Berry, K.; Bass, S.; Linher, K.; Weidman, J.; Khouri, H.; Craven, B.; Bowman, C.; Dodson, R.; Gwinn, M.; Nelson, W.; DeBoy, R.; Kolonay, J.; McClarty, G.; Salzberg, S. L.; Eisen, J.; Fraser, C. M.  
CORPORATE SOURCE: The Institute for Genomic Research, Rockville, MD, 20850, USA  
SOURCE: Nucleic Acids Research (2000), 28(6), 1397-1406

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CODEN: NARHAD; ISSN: 0305-1048

PUBLISHER: Oxford University Press

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The genome sequences of *Chlamydia trachomatis* mouse pneumonitis (MoPn, *Chlamydia muridarum*) strain Nigg (1,069,412 nucleotides) and *Chlamydia pneumoniae* strain AR39 (*Chlamydophila pneumonia*) (1,229,853 nucleotides) were determined using a random shotgun strategy. The MoPn genome exhibited a general conservation of gene order and content with the previously sequenced *C. trachomatis* serovar D. Differences between *C. trachomatis* strains were focused on an .apprx.50-kb "plasticity zone" near the termination origins. In this region MoPn contained 3 copies of a novel gene encoding a >3000-amino-acid toxin homologous to a predicted toxin from *Escherichia coli* O157:H7 but had apparently lost the tryptophan biosynthesis genes found in serovar D in this region. The *C. pneumoniae* AR39 chromosome was >99.9% identical to the previously sequenced *C. pneumoniae* CWL029 genome; however, comparative anal. identified an invertible DNA segment upstream of the uridine kinase gene which was in different orientations in the two genomes. AR39 also contained a novel 4524-nucleotide circular single-stranded (ss)DNA bacteriophage, the first time a virus has been reported infecting *C. pneumoniae*. Although the chlamydial genomes were highly conserved, there were intriguing differences in key nucleotide salvage pathways: *C. pneumoniae* has a uridine kinase gene for dUTP production, MoPn has a uracil phosphorribosyltransferase, while *C. trachomatis* serovar D contains neither gene. Chromosomal comparison revealed that there had been multiple large inversion events since the species divergence of *C. trachomatis* and *C. pneumoniae*, apparently oriented around the axis of the origin of replication and the termination region. The striking synteny of the *Chlamydia* genomes and prevalence of tandemly duplicated genes are evidence of minimal chromosome rearrangement and foreign gene uptake, presumably owing to the ecol. isolation of the obligate intracellular parasites. In the absence of genetic anal., comparative genomics will continue to provide insight into the virulence mechanisms of these important human pathogens.

IT 223709-50-0 261938-30-1

RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)

(amino acid sequence; genome sequences of *Chlamydia trachomatis* MoPn and *Chlamydia pneumoniae* AR39)

REFERENCE COUNT: 41 THERE ARE 41 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 8 OF 10 HCAPLUS COPYRIGHT 2003 ACS on STN

ACCESSION NUMBER: 1999:359660 HCAPLUS

DOCUMENT NUMBER: 131:28638

TITLE: *Chlamydia pneumoniae* genomic sequence and polypeptides and their fragments and uses for the diagnosis, prevention and treatment of infection

INVENTOR(S): Griffais, Remy

PATENT ASSIGNEE(S): Genset, Fr.

SOURCE: PCT Int. Appl., 1912 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English  
 FAMILY ACC. NUM. COUNT: 1  
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9927105	A2	19990603	WO 1998-IB1890	19981120
WO 9927105	A3	19991111		
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
CA 2307846	AA	19990603	CA 1998-2307846	19981120
AU 9911702	A1	19990615	AU 1999-11702	19981120
AU 762606	B2	20030626		
EP 1032674	A2	20000906	EP 1998-954662	19981120
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
BR 9814878	A	20001003	BR 1998-14878	19981120
JP 2002536958	T2	20021105	JP 2000-556579	19981120
US 6559294	B1	20030506	US 1998-198452	19981123
PRIORITY APPLN. INFO.:			FR 1997-14673	A 19971121
			US 1998-107078P	P 19981104
			WO 1998-IB1890	W 19981120

AB The subject of the invention is the genomic sequence and the nucleotide sequences encoding polypeptides of Chlamydia pneumoniae, such as cellular envelope polypeptides, which are secreted or specific, or which are involved in metabolism, in the replication process or in virulence, polypeptides encoded by such sequences, as well as vectors including the said sequences and cells or animals transformed with these vectors. The complete genome sequence of C. pneumoniae strain CM1 (ATCC 1260-VR) is provided, as well as 1296 open reading frames and the deduced amino acid sequences of their protein products. The invention also relates to transcriptional gene products of the Chlamydia pneumoniae genome, such as, for example, antisense and ribozyme mols., which can be used to control growth of the microorganism. The invention also relates to methods of detecting these nucleic acids or polypeptides and kits for diagnosing Chlamydia pneumoniae infection. The invention also relates to a method of selecting compds. capable of modulating bacterial infection and a method for the biosynthesis or biodegrdn. of mols. of interest using the said nucleotide sequences or the said polypeptides. The invention finally comprises, pharmaceutical, in particular vaccine, compns. for the prevention and/or treatment of bacterial, in particular Chlamydia pneumoniae, infections.

IT 225925-21-3

RL: BOC (Biological occurrence); BSU (Biological study, unclassified); PRP (Properties); THU (Therapeutic use); BIOL (Biological study); OCCU (Occurrence); USES (Uses)  
 (amino acid sequence; Chlamydia pneumoniae genomic sequence and polypeptides and their fragments and uses for the diagnosis, prevention and treatment of infection)

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L2 ANSWER 9 OF 10 HCAPLUS COPYRIGHT 2003 ACS on STN  
ACCESSION NUMBER: 1999:216816 HCAPLUS  
DOCUMENT NUMBER: 130:321465  
TITLE: Comparative genomes of *Chlamydia pneumoniae* and  
*C. trachomatis*  
AUTHOR(S): Kalman, Sue; Mitchell, Wayne; Marathe, Rekha;  
Lammel, Claudia; Fan, Jun; Hyman, Richard W.;  
Olinger, Lynn; Grimwood, Jane; Davis, Ronald W.;  
Stephens, Richard S.  
CORPORATE SOURCE: Stanford DNA Sequencing and Technology, Center,  
Stanford University, Stanford, CA, 94305, USA  
SOURCE: Nature Genetics (1999), 21(4), 385-389  
CODEN: NGENEC; ISSN: 1061-4036  
PUBLISHER: Nature America  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB Chlamydia are obligate intracellular eubacteria that are phylogenetically separated from other bacterial divisions. *C. trachomatis* and *C. pneumoniae* are both pathogens of humans but differ in their tissue tropism and spectrum of diseases. *C. pneumoniae* is a newly recognized species of Chlamydia that is a natural pathogen of humans, and causes pneumonia and bronchitis. In the United States, approx. 10% of pneumonia cases and 5% of bronchitis cases are attributed to *C. pneumoniae* infection. Chronic disease may result following respiratory-acquired infection, such as reactive airway disease, adult-onset asthma and potentially lung cancer. In addition, *C. pneumoniae* infection has been associated with atherosclerosis. *C. trachomatis* infection causes trachoma, an ocular infection that leads to blindness, and sexually transmitted diseases such as pelvic inflammatory disease, chronic pelvic pain, ectopic pregnancy and epididymitis. Although relatively little is known about *C. trachomatis* biol., even less is known concerning *C. pneumoniae*. Comparison of the *C. pneumoniae* genome with the *C. trachomatis* genome will provide an understanding of the common biol. processes required for infection and survival in mammalian cells. Genomic differences are implicated in the unique properties that differentiate the two species in disease spectrum. Anal. of the 1,230,230-nt *C. pneumoniae* genome revealed 214 protein-coding sequences not found in *C. trachomatis*, most without homologues to other known sequences. Prominent comparative findings include expansion of a novel family of 21 sequence-variant outer-membrane proteins, conservation of a type-III secretion virulence system, three serine/threonine protein kinases and a pair of paralogous phospholipase-D-like proteins, addnl. purine and biotin biosynthetic capability, a homolog for aromatic amino acid (tryptophan) hydroxylase and the loss of tryptophan biosynthesis genes.

IT 223709-50-0

RL: BSU (Biological study, unclassified); PRP (Properties); BIOL  
(Biological study)  
(amino acid sequence; comparative genomes of *Chlamydia pneumoniae* and *C. trachomatis*)

REFERENCE COUNT: 23 THERE ARE 23 CITED REFERENCES AVAILABLE  
FOR THIS RECORD. ALL CITATIONS AVAILABLE  
IN THE RE FORMAT

L2 ANSWER 10 OF 10 HCAPLUS COPYRIGHT 2003 ACS on STN  
ACCESSION NUMBER: 1998:698040 HCAPLUS  
DOCUMENT NUMBER: 129:326833

Searcher : Shears 308-4994

09/868293

TITLE: Genome sequence of an intracellular pathogen of humans: *Chlamydia trachomatis*  
AUTHOR(S): Stephens, Richard S.; Kalman, Sue; Lammel, Claudia; Fan, Jun; Marathe, Rekha; Aravind, L.; Mitchell, Wayne; Olinger, Lynn; Tatusov, Roman L.; Zhao, Qixun; Koonin, Eugene V.; Davis, Ronald W.  
CORPORATE SOURCE: Program in Infectious Diseases, Univ. California, Berkeley, CA, 94720, USA  
SOURCE: Science (Washington, D. C.) (1998), 282(5389), 754-759  
CODEN: SCIEAS; ISSN: 0036-8075  
PUBLISHER: American Association for the Advancement of Science  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB Anal. of the 1,042,519-base pair *Chlamydia trachomatis* genome revealed unexpected features related to the complex biol. of chlamydiae. Although chlamydiae lack many biosynthetic capabilities, they retain functions for performing key steps and interconversions of metabolites obtained from their mammalian host cells. Numerous potential virulence-associated proteins also were characterized. Several eukaryotic chromatin-associated domain proteins were identified, suggesting a eukaryotic-like mechanism for chlamydial nucleoid condensation and decondensation. The phylogenetic mosaic of chlamydial genes, including a large number of genes with phylogenetic origins from eukaryotes, implies a complex evolution for adaptation to obligate intracellular parasitism.  
IT 215102-41-3  
RL: PRP (Properties)  
(amino acid sequence; genome sequence of *Chlamydia trachomatis*)  
REFERENCE COUNT: 58 THERE ARE 58 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

(FILE 'HCAPLUS' ENTERED AT 14:39:39 ON 30 OCT 2003)  
L5 2181 SEA FILE=HCAPLUS ABB=ON PLU=ON (CHLAMYDIA# OR TRACHOMAT ? OR P38016 OR P 38016) AND INFECTION  
L6 5 SEA FILE=HCAPLUS ABB=ON PLU=ON L5 AND (RIBOSOM?(S) ("L7" (W) "L12" OR "L7L12"))

L7 3 L6 NOT L2

L7 ANSWER 1 OF 3 HCAPLUS COPYRIGHT 2003 ACS on STN  
ACCESSION NUMBER: 2002:778631 HCAPLUS  
DOCUMENT NUMBER: 137:290038  
TITLE: Nucleic acids and proteins from *Chlamydia trachomatis* and methods for treatment and diagnosis of *chlamydial infection*  
INVENTOR(S): Bhatia, Ajay; Probst, Peter  
PATENT ASSIGNEE(S): Corixa Corporation, USA  
SOURCE: U.S. Pat. Appl. Publ., 42 pp., Cont.-in-part of U.S. Ser. No. 841,260.  
CODEN: USXXCO  
DOCUMENT TYPE: Patent  
LANGUAGE: English

Searcher : Shears 308-4994

## PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 2002146776 A1		20021010	US 2001-7693	20011205
PRIORITY APPLN. INFO.:			US 2000-PV198853	20000421
			US 2000-PV219752	20000720
			US 2001-841260	20010423

AB Nucleic acid and protein compds. and methods for the diagnosis and treatment of **chlamydial infection** are disclosed. The compds. provided include polypeptides that contain at least one antigenic portion of a **Chlamydia** antigen and genomic DNA sequences encoding such polypeptides from **C. trachomatis** serovar E and serovar D. Pharmaceutical compns. and vaccines comprising such polypeptides or DNA sequences are also provided, together with antibodies directed against such polypeptides. Diagnostic kits containing such polypeptides or DNA sequences and a suitable detection reagent may be used for the detection of **chlamydial infection** in patients and in biol. samples. The present invention claims SEQ IDs 1-48, 80-109, and 114-157, but the Sequence Listing was not made available on publication of the patent application.

IT Chaperonins  
 RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
 (GroEL; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of **chlamydial infection**)

IT Chaperonins  
 RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
 (GroES; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of **chlamydial infection**)

IT Ribosomal proteins  
 RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
 (L16; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of **chlamydial infection**)

IT Ribosomal proteins  
 RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
 (L1; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of **chlamydial infection**)

IT Ribosomal proteins  
 RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
 (L22; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of **chlamydial infection**)

IT Ribosomal proteins

RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(L2; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)

IT Ribosomal proteins

RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(L3; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)

IT Ribosomal proteins

RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(L4; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)

IT Ribosomal proteins

RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(L7/L12; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)

IT Ribosomal proteins

RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(S19; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)

IT Ribosomal proteins

RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(S9; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)

IT Proteins

RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(SWIB; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)

IT Proteins

RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(TSA; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)

IT Gene, microbial

RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST

(Analytical study); BIOL (Biological study); USES (Uses)  
(accC; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)

IT Infection

(bacterial; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)

IT Antigen-presenting cell

CD4-positive T cell

CD8-positive T cell

(chlamydial antigen-specific; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)

IT Gene, microbial

RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(clpB; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)

IT Gene, microbial

RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(dag2; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)

IT Nucleic acid hybridization

(diagnostic assay; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)

IT Immunoassay

Test kits

(diagnostic; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)

IT Gene, microbial

RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(dnaK; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)

IT Gene, microbial

RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(fabI; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)

IT Gene, microbial

RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(fliA; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)

- chlamydial infection)**
- IT Gene, microbial  
 RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
     (*ftsH*; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of **chlamydial infection**)
- IT Gene, microbial  
 RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
     (*grpE*; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of **chlamydial infection**)
- IT Gene, microbial  
 RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
     (*gyrA-2*; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of **chlamydial infection**)
- IT Gene, microbial  
 RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
     (*hctA*; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of **chlamydial infection**)
- IT Gene, microbial  
 RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
     (*lipA*; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of **chlamydial infection**)
- IT Gene, microbial  
 RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
     (*lpdA*; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of **chlamydial infection**)
- IT Gene, microbial  
 RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
     (*ltuA*; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of **chlamydial infection**)
- IT Gene, microbial  
 RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
     (*mesJ*; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of **chlamydial infection**)
- IT Gene, microbial

RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(mhpA; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)

IT Gene, microbial

RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(mreB; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)

IT Gene, microbial

RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(mutS; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)

IT Gene, microbial

RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(nrdA; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)

IT Gene, microbial

RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(nrdB; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)

IT Antibacterial agents

**Chlamydia trachomatis**

DNA sequences

Molecular cloning

Protein sequences

Vaccines

(nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)

IT Antigens

Gene, microbial

Proteins

RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)

IT Probes (nucleic acid)

RL: ARG (Analytical reagent use); DGN (Diagnostic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)

- IT Antibodies  
RL: ARG (Analytical reagent use); DGN (Diagnostic use); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)
- IT Fusion proteins (chimeric proteins)  
RL: DGN (Diagnostic use); THU (Therapeutic use); BIOL (Biological study); USES (Uses)  
(nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)
- IT Gene, microbial  
RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(*pckA*; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)
- IT Gene, microbial  
RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(*pepA*; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)
- IT Gene, microbial  
RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(*pgi*; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)
- IT Gene, microbial  
RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(*phoH*; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)
- IT Gene, microbial  
RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(*pmpB*; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)
- IT Gene, microbial  
RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(*pmpH*; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)
- IT Gene, microbial  
RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)

(Analytical study); BIOL (Biological study); USES (Uses)  
(pmpI; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)

- IT Gene, microbial  
RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(pnp; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)
- IT Gene, microbial  
RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(prfB; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)
- IT Gene, microbial  
RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(ribF; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)
- IT Gene, microbial  
RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(secE; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)
- IT Gene, microbial  
RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(sfhB; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)
- IT T cell (lymphocyte)  
(stimulation and/or expansion of chlamydial antigen-specific; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)
- IT Gene, microbial  
RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(truB; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)
- IT Gene, microbial  
RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(tyrS; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)

- IT Gene, microbial  
Gene, microbial  
RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(yaeI; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)
- IT Gene, microbial  
RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(ybcL; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)
- IT Gene, microbial  
RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(ychF; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)
- IT Gene, microbial  
RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(ydhO; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)
- IT Gene, microbial  
RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(ygcA; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)
- IT Gene, microbial  
RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(yscC; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)
- IT Gene, microbial  
RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(yscU; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)
- IT 9001-64-3, Malate dehydrogenase 9014-56-6, Glycogen synthase  
39369-30-7, RRNA methylase  
RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
(nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of chlamydial infection)

- IT 9000-83-3, ATPase  
 RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
 (phoH; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of **chlamydial infection**)
- IT 9040-57-7, Ribonucleotide reductase  
 RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
 (small and large subunits; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of **chlamydial infection**)
- IT 9014-24-8, RNA polymerase  
 RL: ANT (Analyte); BSU (Biological study, unclassified); DGN (Diagnostic use); PRP (Properties); THU (Therapeutic use); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
 ( $\beta$ ; nucleic acids and proteins from **Chlamydia trachomatis** and methods for treatment and diagnosis of **chlamydial infection**)

L7 ANSWER 2 OF 3 HCAPLUS COPYRIGHT 2003 ACS on STN  
 ACCESSION NUMBER: 1999:577186 HCAPLUS  
 DOCUMENT NUMBER: 131:211217  
 TITLE: Identification of immunoreactive proteins of **Chlamydia trachomatis** by Western blot analysis of a two-dimensional electrophoresis map with patient sera  
 AUTHOR(S): Sanchez-Campillo, Maria; Bini, Luca; Comanducci, Maurizio; Raggiaschi, Roberto; Marzocchi, Barbara; Pallini, Vitaliano; Ratti, Giulio  
 CORPORATE SOURCE: IRIS Reserach Center, Siena, I-53100, Italy  
 SOURCE: Electrophoresis (1999), 20(11), 2269-2279  
 CODEN: ELCTDN; ISSN: 0173-0835  
 PUBLISHER: Wiley-VCH Verlag GmbH  
 DOCUMENT TYPE: Journal  
 LANGUAGE: English  
 AB Western blots of two-dimensional electrophoretic maps of proteins from **Chlamydia trachomatis** were probed with sera from 17 seropos. patients with genital inflammatory disease. Immunoblot patterns (comprising 28 to 2 spots, average 14.8) were different for each patient; however, antibodies against a spot-cluster due to the **chlamydia**-specific antigen outer membrane protein-2 (OMP2) were observed in all sera. The next most frequent group of antibodies (15/17; 88%) recognized the hsp60 GroEL-like protein, described as immunopathogenic in **chlamydial infections**. Reactivity to the major surface-exposed and variable antigen major outer membrane protein (MOMP) was observed at a relatively lower frequency (13/17; 76%). The hsp70 DnaK-like protein was also frequently recognized (11/17; 64.7%) in this patient group. Besides the above confirmatory findings, the study detected several new immunoreactive proteins, with frequencies ranging from 11/17 to 1/17. Some were characterized also by N-terminal amino acid sequencing and homol. searches. Amongst these were a novel outer membrane protein (OmpB) and, interestingly, five conserved bacterial proteins: four (23%) sera reacted with the RNA polymerase alpha-subunit, five (29%)

recognized the **ribosomal** protein S1, eight (47%) the protein elongation factor EF-Tu, seven (41%) a putative stress-induced protease of the HtrA family, and seven sera (41%) the **ribosomal** protein L7/L12. Homologs of the last two proteins were shown to confer protective immunity in other bacterial **infections**. The data show that immunological sensitization processes commonly thought to play a role in **chlamydial** pathogenicity may be sustained not only by the hsp60 GroEL-like protein, but also by other conserved bacterial antigens, some of which may be also considered as potential vaccine candidates.

## IT Proteins, specific or class

RL: ANT (Analyte); BOC (Biological occurrence); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); OCCU (Occurrence)  
 (DNAK-like; identification of immunoreactive proteins of **Chlamydia trachomatis** by Western blot anal. of 2-D electrophoresis map with patient sera)

## IT Peptides, analysis

Proteins, specific or class

RL: ANT (Analyte); BOC (Biological occurrence); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); OCCU (Occurrence)  
 (EF-Tu; identification of immunoreactive proteins of **Chlamydia trachomatis** by Western blot anal. of 2-D electrophoresis map with patient sera)

## IT Proteins, specific or class

RL: ANT (Analyte); BOC (Biological occurrence); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); OCCU (Occurrence)  
 (GTP-binding; identification of immunoreactive proteins of **Chlamydia trachomatis** by Western blot anal. of 2-D electrophoresis map with patient sera)

## IT Proteins, specific or class

RL: ANT (Analyte); BOC (Biological occurrence); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); OCCU (Occurrence)  
 (GroEL-like; identification of immunoreactive proteins of **Chlamydia trachomatis** by Western blot anal. of 2-D electrophoresis map with patient sera)

## IT Proteins, specific or class

Ribosomal proteins

RL: ANT (Analyte); BOC (Biological occurrence); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); OCCU (Occurrence)  
 (L7/12; identification of immunoreactive proteins of **Chlamydia trachomatis** by Western blot anal. of 2-D electrophoresis map with patient sera)

## IT Proteins, specific or class

RL: ANT (Analyte); BOC (Biological occurrence); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); OCCU (Occurrence)  
 (MMOMP; identification of immunoreactive proteins of **Chlamydia trachomatis** by Western blot anal. of 2-D electrophoresis map with patient sera)

## IT Proteins, specific or class

RL: ANT (Analyte); BOC (Biological occurrence); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological

study); OCCU (Occurrence)  
 (OMP (outer membrane protein); identification of immunoreactive proteins of **Chlamydia trachomatis** by Western blot anal. of 2-D electrophoresis map with patient sera)

IT Proteins, specific or class  
 RL: ANT (Analyte); BOC (Biological occurrence); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); OCCU (Occurrence)  
 (OMP2; identification of immunoreactive proteins of **Chlamydia trachomatis** by Western blot anal. of 2-D electrophoresis map with patient sera)

IT Ribozymes  
 RL: ANT (Analyte); BOC (Biological occurrence); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); OCCU (Occurrence)  
 (P,  $\alpha$ -chain; S1; identification of immunoreactive proteins of **Chlamydia trachomatis** by Western blot anal. of 2-D electrophoresis map with patient sera)

IT Ribosomal proteins  
 RL: ANT (Analyte); BOC (Biological occurrence); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); OCCU (Occurrence)  
 (S1; identification of immunoreactive proteins of **Chlamydia trachomatis** by Western blot anal. of 2-D electrophoresis map with patient sera)

IT Gene, microbial  
 RL: BSU (Biological study, unclassified); BIOL (Biological study)  
 (dnaK; identification of immunoreactive proteins of **Chlamydia trachomatis** by Western blot anal. of 2-D electrophoresis map with patient sera)

IT Gene, microbial  
 RL: BSU (Biological study, unclassified); BIOL (Biological study)  
 (groEL1; identification of immunoreactive proteins of **Chlamydia trachomatis** by Western blot anal. of 2-D electrophoresis map with patient sera)

IT **Chlamydia trachomatis**  
 (identification of immunoreactive proteins of **Chlamydia trachomatis** by Western blot anal. of 2-D electrophoresis map with patient sera)

IT Gene, microbial  
 RL: BSU (Biological study, unclassified); BIOL (Biological study)  
 (jtrA; identification of immunoreactive proteins of **Chlamydia trachomatis** by Western blot anal. of 2-D electrophoresis map with patient sera)

IT Gene, microbial  
 RL: BSU (Biological study, unclassified); BIOL (Biological study)  
 (omcB; identification of immunoreactive proteins of **Chlamydia trachomatis** by Western blot anal. of 2-D electrophoresis map with patient sera)

IT Gene, microbial  
 RL: BSU (Biological study, unclassified); BIOL (Biological study)  
 (ompA; identification of immunoreactive proteins of **Chlamydia trachomatis** by Western blot anal. of 2-D electrophoresis map with patient sera)

IT Gene, microbial  
 RL: BSU (Biological study, unclassified); BIOL (Biological study)  
 (ompB; identification of immunoreactive proteins of **Chlamydia trachomatis** by Western blot anal. of

2-D electrophoresis map with patient sera)

IT Gene, microbial  
 RL: BSU (Biological study, unclassified); BIOL (Biological study)  
 (ompA; identification of immunoreactive proteins of  
*Chlamydia trachomatis* by Western blot anal. of  
 2-D electrophoresis map with patient sera)

IT Gene, microbial  
 RL: BSU (Biological study, unclassified); BIOL (Biological study)  
 (pepA; identification of immunoreactive proteins of  
*Chlamydia trachomatis* by Western blot anal. of  
 2-D electrophoresis map with patient sera)

IT Gene, microbial  
 RL: BSU (Biological study, unclassified); BIOL (Biological study)  
 (r17; identification of immunoreactive proteins of  
*Chlamydia trachomatis* by Western blot anal. of  
 2-D electrophoresis map with patient sera)

IT Gene, microbial  
 RL: BSU (Biological study, unclassified); BIOL (Biological study)  
 (rpoA; identification of immunoreactive proteins of  
*Chlamydia trachomatis* by Western blot anal. of  
 2-D electrophoresis map with patient sera)

IT Gene, microbial  
 RL: BSU (Biological study, unclassified); BIOL (Biological study)  
 (rs1; identification of immunoreactive proteins of  
*Chlamydia trachomatis* by Western blot anal. of  
 2-D electrophoresis map with patient sera)

IT Gene, microbial  
 RL: BSU (Biological study, unclassified); BIOL (Biological study)  
 (tufA; identification of immunoreactive proteins of  
*Chlamydia trachomatis* by Western blot anal. of  
 2-D electrophoresis map with patient sera)

IT Electrophoresis  
 (two-dimensional; identification of immunoreactive proteins of  
*Chlamydia trachomatis* by Western blot anal. of  
 2-D electrophoresis map with patient sera)

IT Gene, microbial  
 RL: BSU (Biological study, unclassified); BIOL (Biological study)  
 (ychF; identification of immunoreactive proteins of  
*Chlamydia trachomatis* by Western blot anal. of  
 2-D electrophoresis map with patient sera)

IT 9031-94-1, Aminopeptidase  
 RL: ANT (Analyte); BOC (Biological occurrence); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); OCCU (Occurrence)  
 (identification of immunoreactive proteins of *Chlamydia trachomatis* by Western blot anal. of 2-D electrophoresis map with patient sera)

IT 9001-92-7, Proteinase  
 RL: ANT (Analyte); BOC (Biological occurrence); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); OCCU (Occurrence)  
 (stress-induced; identification of immunoreactive proteins of *Chlamydia trachomatis* by Western blot anal. of 2-D electrophoresis map with patient sera)

REFERENCE COUNT: 32 THERE ARE 32 CITED REFERENCES AVAILABLE  
 FOR THIS RECORD. ALL CITATIONS AVAILABLE  
 IN THE RE FORMAT

09/868293

L7 ANSWER 3 OF 3 HCPLUS COPYRIGHT 2003 ACS on STN  
ACCESSION NUMBER: 1990:585611 HCPLUS  
DOCUMENT NUMBER: 113:185611  
TITLE: Cloning and characterization of RNA polymerase  
core subunits of **Chlamydia**  
**trachomatis** by using the polymerase  
chain reaction  
AUTHOR(S): Engel, Joanne N.; Pollack, Jonathan; Malik,  
Fady; Ganem, Don  
CORPORATE SOURCE: Dep. Microbiol. Immunol., Univ. California, San  
Francisco, CA, 94143, USA  
SOURCE: Journal of Bacteriology (1990), 172(10), 5732-41  
CODEN: JOBAAY; ISSN: 0021-9193  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Taking advantage of sequence conservation of portions of the  $\alpha$ ,  $\beta$ , and  $\beta'$  subunits of RNA polymerase of bacteria and plant chloroplasts, degenerate oligonucleotides were designed corresponding to these domains and as primers in a polymerase chain reaction to amplify DNA sequences from the **chlamydial** genome. The polymerase chain reaction products were used as a probe to recover the genomic fragments encoding the  $\beta$  subunit and the 5' portion of the  $\beta'$  subunit from a library of cloned murine C. **trachomatis** DNA. Similar attempts to recover the  $\alpha$  subunit were unsuccessful. Sequence anal. demonstrated that the  $\beta$  subunit of RNA polymerase was located between codes encoding the L7/L12 ribosomal protein and the  $\beta'$  subunit of RNA polymerase; this organization is reminiscent of the rpoBC operon of Escherichia coli. The C. **trachomatis**  $\beta$  subunit overproduced in E. coli was used as an antigen in rabbits to make a polyclonal antibody to this subunit. Although this polyclonal antibody specifically immunopptd. the  $\beta$  subunit from **Chlamydia**-infected cells, it did not immunoppt. core or holoenzyme. Immunoblots with this antibody demonstrated that the  $\beta$  subunit appeared early in infection.

IT **Chlamydia trachomatis**  
(RNA polymerase  $\beta$ -subunit of, gene for, cloning and  
expression in infected cells of)  
IT Gene and Genetic element, microbial  
RL: BIOL (Biological study)  
(for RNA polymerase  $\beta$ -subunit, of **Chlamydia**  
**trachomatis**, cloning and expression in infected cells of)  
IT Molecular cloning  
(of RNA polymerase  $\beta$ -subunit gene, of **Chlamydia**  
**trachomatis**, with polymerase chain reaction)  
IT 9014-24-8, RNA polymerase  
RL: BIOL (Biological study)  
( $\beta$ -subunit, gene for, of **Chlamydia**  
**trachomatis**, cloning and expression in infected cells of)

(FILE 'MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH,  
JICST-EPLUS, JAPIO' ENTERED AT 14:42:13 ON 30 OCT 2003)

L8 8 S L6  
L9 3 DUP REM L8 (5 DUPLICATES REMOVED)

L9 ANSWER 1 OF 3 MEDLINE on STN DUPLICATE 1  
ACCESSION NUMBER: 1999420874 MEDLINE  
DOCUMENT NUMBER: 99420874 PubMed ID: 10493131

09/868293

TITLE: Identification of immunoreactive proteins of *Chlamydia trachomatis* by Western blot analysis of a two-dimensional electrophoresis map with patient sera.

AUTHOR: Sanchez-Campillo M; Bini L; Comanducci M; Raggiaschi R; Marzocchi B; Pallini V; Ratti G

CORPORATE SOURCE: IRIS Research Centre, Chiron Vaccines, Siena, Italy.

SOURCE: ELECTROPHORESIS, (1999 Aug) 20 (11) 2269-79.  
Journal code: 8204476. ISSN: 0173-0835.

PUB. COUNTRY: GERMANY: Germany, Federal Republic of

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199912

ENTRY DATE: Entered STN: 20000113  
Last Updated on STN: 20000113  
Entered Medline: 19991209

AB Western blots of two-dimensional electrophoretic maps of proteins from *Chlamydia trachomatis* were probed with sera from 17 seropositive patients with genital inflammatory disease. Immunoblot patterns (comprising 28 to 2 spots, average 14.8) were different for each patient; however, antibodies against a spot-cluster due to the *chlamydia*-specific antigen outer membrane protein-2 (OMP2) were observed in all sera. The next most frequent group of antibodies (15/17; 88%) recognized the hsp60 GroEL-like protein, described as immunopathogenic in *chlamydial infections*. Reactivity to the major surface-exposed and variable antigen major outer membrane protein (MOMP) was observed at a relatively lower frequency (13/17; 76%). The hsp70 DnaK-like protein was also frequently recognized (11/17; 64.7%) in this patient group. Besides the above confirmatory findings, the study detected several new immunoreactive proteins, with frequencies ranging from 11/17 to 1/17. Some were characterized also by N-terminal amino acid sequencing and homology searches. Amongst these were a novel outer membrane protein (OmpB) and, interestingly, five conserved bacterial proteins: four (23%) sera reacted with the RNA polymerase alpha-subunit, five (29%) recognized the ribosomal protein S1, eight (47%) the protein elongation factor EF-Tu, seven (41%) a putative stress-induced protease of the HtrA family, and seven sera (41%) the ribosomal protein L7/L12. Homologs of the last two proteins were shown to confer protective immunity in other bacterial *infections*. The data show that immunological sensitization processes commonly thought to play a role in *chlamydial* pathogenicity may be sustained not only by the hsp60 GroEl-like protein, but also by other conserved bacterial antigens, some of which may be also considered as potential vaccine candidates.

L9 ANSWER 2 OF 3 MEDLINE on STN DUPLICATE 2  
ACCESSION NUMBER: 91008945 MEDLINE  
DOCUMENT NUMBER: 91008945 PubMed ID: 2211507  
TITLE: Cloning and characterization of RNA polymerase core subunits of *Chlamydia trachomatis*  
by using the polymerase chain reaction.  
AUTHOR: Engel J N; Pollack J; Malik F; Ganem D  
CORPORATE SOURCE: Department of Microbiology, University of California,  
San Francisco, 94143.

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CONTRACT NUMBER: AI24436 (NIAID)  
SOURCE: JOURNAL OF BACTERIOLOGY, (1990 Oct) 172 (10) 5732-41.  
Journal code: 2985120R. ISSN: 0021-9193.  
PUB. COUNTRY: United States  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Priority Journals.  
ENTRY MONTH: 199011  
ENTRY DATE: Entered STN: 19910117  
Last Updated on STN: 19980206  
Entered Medline: 19901114

AB Taking advantage of sequence conservation of portions of the alpha, beta, and beta' subunits of RNA polymerase of bacteria and plant chloroplasts, we have designed degenerate oligonucleotides corresponding to these domains and used these synthetic DNA sequences as primers in a polymerase chain reaction to amplify DNA sequences from the **chlamydial** genome. The polymerase chain reaction products were used as a probe to recover the genomic fragments encoding the beta subunit and the 5' portion of the beta' subunit from a library of cloned murine **Chlamydia trachomatis** DNA. Similar attempts to recover the alpha subunit were unsuccessful. Sequence analysis demonstrated that the beta subunit of RNA polymerase was located between genes encoding the L7/L12 ribosomal protein and the beta' subunit of RNA polymerase; this organization is reminiscent of the rpoBC operon of Escherichia coli. The **C. trachomatis** beta subunit overproduced in E. coli was used as an antigen in rabbits to make a polyclonal antibody to this subunit. Although this polyclonal antibody specifically immunoprecipitated the beta subunit from **Chlamydia**-infected cells, it did not immunoprecipitate core or holoenzyme. Immunoblots with this antibody demonstrated that the beta subunit appeared early in infection.

L9 ANSWER 3 OF 3 JAPIO (C) 2003 JPO on STN  
ACCESSION NUMBER: 2001-286295 JAPIO  
TITLE: ANTIBODY FOR DETECTING **CHLAMYDIA TRACHOMATIS**  
INVENTOR: RAMAN MONZUURU; ETO TAKASHI  
PATENT ASSIGNEE(S): ASAHI KASEI CORP  
PATENT INFORMATION:

PATENT NO	KIND	DATE	ERA	MAIN IPC
JP 2001286295	A	20011016	Heisei	C12P021-08

APPLICATION INFORMATION

STN FORMAT: JP 2001-24749 20010131  
ORIGINAL: JP2001024749 Heisei  
PRIORITY APPLN. INFO.: JP 2000-62685 20000131  
SOURCE: PATENT ABSTRACTS OF JAPAN (CD-ROM), Unexamined Applications, Vol. 2001

AN 2001-286295 JAPIO  
AB PROBLEM TO BE SOLVED: To provide a method for specifically and rapidly with high sensitivity detecting a microorganism belonging to **Chlamydia trachomatis**, an antibody for detection used for detecting the microorganism, a reagent kit for detection and a method for producing the antibody for detection used for

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detecting the microorganism.

SOLUTION: This antibody is an antibody against **ribosomal protein** of the microorganism belonging to **Chlamydia trachomatis** and reacts specifically with the microorganism. The method for detecting the microorganism belonging to **Chlamydia trachomatis** by the use of the antibody, the reagent kit for detection and the method for producing the antibody are provided. As the **ribosomal protein**, the **ribosomal protein L7/L12** protein is illustrated and used for detecting the **infection** of the causal microorganism of STD(sexually transmitted diseases).

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(FILE 'MEDLINE' ENTERED AT 14:44:18 ON 30 OCT 2003)

L10 6760 SEA FILE=MEDLINE ABB=ON PLU=ON "CHLAMYDIA TRACHOMATIS"/

CT

L11 6886 SEA FILE=MEDLINE ABB=ON PLU=ON "RIBOSOMAL PROTEINS"/CT

L12 6 SEA FILE=MEDLINE ABB=ON PLU=ON L10 AND L11

L12 ANSWER 1 OF 6 MEDLINE on STN

AN 1999057063 MEDLINE

TI Correlation between chlamydial infection and autoimmune response: molecular mimicry between RNA polymerase major sigma subunit from Chlamydia trachomatis and human L7.

AU Hemmerich P; Neu E; Macht M; Peter H H; Krawinkel U; von Mikecz A  
SO EUROPEAN JOURNAL OF IMMUNOLOGY, (1998 Nov) 28 (11) 3857-66.

Journal code: 1273201. ISSN: 0014-2980.

AB L7 is one of the ribosomal proteins frequently targeted by autoantibodies in rheumatic autoimmune diseases. A computer search revealed a region within the immunodominant epitope of L7 (peptide II) that is highly homologous to amino acid sequence 264-286 of the RNA polymerase major sigma factor of the eubacterium Chlamydia trachomatis. Anti-L7 autoantibodies affinity purified from the immunodominant epitope were able to recognize this sequence as they reacted with purified recombinant sigma factor. Immunofluorescence labeling experiments on C. trachomatis lysates revealed a punctate staining pattern of numerous spots when incubated with the affinity-purified anti-peptide II autoantibodies. Binding of autoantibodies to peptide II was inhibited by the homologous sigma peptide. This is the first demonstration of epitope mimicry between a human and a chlamydial protein on the level of B cells. Antibody screening revealed a significant correlation between the presence of anti-L7 autoantibodies and C. trachomatis infection in patients with systemic lupus erythematosus and mixed connective tissue disease. Our results suggest that molecular mimicry is involved in the initiation of anti-L7 autoantibody response and may represent a first glance into the immunopathology of Chlamydia with respect to systemic rheumatic diseases.

L12 ANSWER 2 OF 6 MEDLINE on STN

AN 95247702 MEDLINE

TI Chlamydia trachomatis RNA polymerase alpha subunit: sequence and structural analysis.

AU Gu L; Wenman W M; Remacha M; Meuser R; Coffin J; Kaul R  
SO JOURNAL OF BACTERIOLOGY, (1995 May) 177 (9) 2594-601.

Journal code: 2985120R. ISSN: 0021-9193.

AB We describe the cloning and sequence analysis of the region

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surrounding the gene for the alpha subunit of RNA polymerase from Chlamydia trachomatis. This region contains genes for proteins in the order SecY, S13, S11, alpha, and L17, which are equivalent to Escherichia coli and Bacillus subtilis r proteins. The incorporation of chlamydial alpha subunit protein into the E. coli RNA polymerase holoenzyme rather than its truncated variant lacking the amino terminus suggests the existence of structural conservation among alpha subunits from distantly related genera.

L12 ANSWER 3 OF 6 MEDLINE on STN  
AN 94042887 MEDLINE  
TI Cloning and characterization of the RNA polymerase alpha-subunit operon of Chlamydia trachomatis.  
AU Tan M; Klein R; Grant R; Ganem D; Engel J  
SO JOURNAL OF BACTERIOLOGY, (1993 Nov) 175 (22) 7150-9.  
Journal code: 2985120R. ISSN: 0021-9193.  
AB We have cloned the chlamydial operon that encodes the initiation factor IF1, the ribosomal proteins L36, S13, and S11, and the alpha subunit of RNA polymerase. The genes for S11 and alpha are closely linked in Escherichia coli, Bacillus subtilis, and plant chloroplast genomes, and this arrangement is conserved in Chlamydia spp. The S11 ribosomal protein gene potentially encodes a protein of 125 amino acids with 41 to 42% identity over its entire length to its E. coli and B. subtilis homologs; the gene encoding the alpha subunit specifies a protein of 322 amino acids with 25 to 30% identity over its entire length to its E. coli and B. subtilis homologs. In a T7-based expression system in E. coli, the chlamydial alpha gene directed the synthesis of a 36-kDa protein. Mapping of the chlamydial mRNA transcript by RNase protection studies and by a combination of reverse transcription and the polymerase chain reaction demonstrates that IF1, L36, S13, S11, and alpha are transcribed as a polycistronic transcript.

L12 ANSWER 4 OF 6 MEDLINE on STN  
AN 92203999 MEDLINE  
TI The gene for the S7 ribosomal protein of Chlamydia trachomatis: characterization within the chlamydial str operon.  
AU Wagar E A; Pang M  
SO MOLECULAR MICROBIOLOGY, (1992 Feb) 6 (3) 327-35.  
Journal code: 8712028. ISSN: 0950-382X.  
AB The prokaryotic ribosomal operon, str, contains open reading frames for the two elongation factors, elongation factor G (EF-G) and elongation factor Tu (EF-Tu), and ribosomal proteins S7 and S12. The DNA sequence and predicted amino acid sequence for S7 from Chlamydia trachomatis are presented and compared with homologues from other prokaryotes. Also, the relationship of the S7 gene to the open reading frames for ribosomal protein S12 and EF-G is described. Significant amino acid homology is also noted when the amino-terminal sequence of chlamydial EF-G is compared with the cytoplasmic tetracycline resistance factors, tetM and tetO, from streptococci and Campylobacter jejuni. Related findings and possible resistance mechanisms for the newly recognized tetracycline-resistant clinical isolates of C. trachomatis are discussed.

L12 ANSWER 5 OF 6 MEDLINE on STN  
AN 92138612 MEDLINE  
TI Cloning and sequence analysis of the Chlamydia trachomatis spc

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ribosomal protein gene cluster.  
AU Kaul R; Gray G J; Koehncke N R; Gu L J  
SO JOURNAL OF BACTERIOLOGY, (1992 Feb) 174 (4) 1205-12.  
Journal code: 2985120R. ISSN: 0021-9193.  
AB We identified and sequenced a segment of Chlamydia trachomatis chromosomal DNA that shows homology to the Escherichia coli spc and distal region of the S10 ribosomal protein (r-protein) operons. Its sequence revealed a high degree of nucleotide and operon context conservation with the E. coli r-protein genes. The C. trachomatis spec operon contains the r-protein genes for L14, L24, L5, S8, L6, L18, S5, L15, and Sec Y along with the genes for r-proteins L16, L29, and S17 of the S10 operon. The two operons are separated by a 16-bp intragenic region which contains no transcription signals. However, a putative promoter for the transcription of the spc operon was found 162 nucleotides upstream of the CtrL14e start site; it revealed significant homology to the E. coli consensus promoter sequences. Interestingly, our results indicate the absence of any structure resembling an EcoS8 regulatory target site on C. trachomatis spc mRNA in spite of significant amino acid identity between E. coli and C. trachomatis r-proteins. Also, the intrinsic aminoglycoside resistance in C. trachomatis is unlikely to be mediated by CtrL6e since E. coli expressing CtrL6e remained susceptible to gentamicin (MIC less than 0.5 micrograms/ml).

L12 ANSWER 6 OF 6 MEDLINE on STN  
AN 91154120 MEDLINE  
TI Isolation and molecular characterization of the ribosomal protein L6 homolog from Chlamydia trachomatis.  
AU Gray G J; Kaul R; Roy K L; Wenman W M  
SO JOURNAL OF BACTERIOLOGY, (1991 Mar) 173 (5) 1663-9.  
Journal code: 2985120R. ISSN: 0021-9193.  
AB The cloning of a Chlamydia trachomatis eukaryotic cell-binding protein reported earlier from our laboratory (R. Kaul, K. L. Roy, and W. M. Wenman, J. Bacteriol. 169:5152-5156, 1987) represents an artifact generated by nonspecific recombination of chromosomal DNA fragments. However, the amino terminus of this plasmid-encoded fusion product demonstrated significant homology to Escherichia coli ribosomal protein L6. By using a 458-bp PstI-HindIII fragment of recombinant pCT161/18 (representing the 5' end of the cloned gene), we isolated and characterized a C. trachomatis homolog of the ribosomal protein L6 gene of E. coli. Sequence analysis of an 1,194-bp EcoRI-SacI fragment that encodes chlamydial L6 (designated CtaL6e) revealed a 552-bp open reading frame comprising 183 amino acids and encodes a protein with a molecular weight of 19,839. Interestingly, complete gene homology between C. trachomatis serovars L2 and J, each of which exists as a single copy per genome, was observed. Expression of a plasmid-encoded gene product is dependent on the lac promoter, since no product was obtained if the open reading frame was oriented in opposition to the lac promoter. Immunoblotting of purified ribosomes revealed functional, as well as antigenic, homology between the E. coli and C. trachomatis ribosomal L6 proteins.

(FILE 'USPATFULL' ENTERED AT 14:44:58 ON 30 OCT 2003)  
L13 2959 SEA FILE=USPATFULL ABB=ON PLU=ON (CHLAMYDIA# OR TRACHOMAT? OR P38016 OR P 38016) (L) INFECTION  
L14 23 SEA FILE=USPATFULL ABB=ON PLU=ON L13(L) (RIBOSOM?(S) ("L7 "(W) "L12" OR "L7L12"))

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L15 23 SEA FILE=USPATFULL ABB=ON PLU=ON L14(L) (TREAT? OR  
THERAP? OR PREVENT?)

L15 ANSWER 1 OF 23 USPATFULL on STN

ACCESSION NUMBER: 2003:285242 USPATFULL

TITLE: Crystals of the large ribosomal subunit

INVENTOR(S): Steitz, Thomas A., Branford, CT, United States

Moore, Peter B., North Haven, CT, United States

Ban, Nenad, Zurich, SWITZERLAND

Nissen, Poul, Aarhus, DENMARK

Hansen, Jeffrey, New Haven, CT, United States

PATENT ASSIGNEE(S): Yale University, New Haven, CT, United States  
(U.S. corporation)

W3

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6638908	B1	20031028
APPLICATION INFO.:	US 2000-635708		20000809 (9)
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	GRANTED		
PRIMARY EXAMINER:	Allen, Marianne P.		
ASSISTANT EXAMINER:	Mahatan, C.		
LEGAL REPRESENTATIVE:	Testa, Hurwitz & Thibeault, LLP		
NUMBER OF CLAIMS:	28		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	62 Drawing Figure(s); 23 Drawing Page(s)		
LINE COUNT:	3884		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides methods for producing high resolution crystals of ribosomes and ribosomal subunits as well as the crystals produced by such methods. The x-ray diffraction patterns of the crystals provided by the present invention are of sufficiently high resolution for determining the three-dimensional structure of ribosomes and ribosomal subunits, for identifying ligand binding sites on ribosomes and ribosomal subunits, and for molecular modeling of ligands which interact with ribosomes and ribosomal subunits. The present invention provides methods for identifying ribosome-related ligands and methods for designing ligands with specific ribosome-binding properties. Thus, the methods of the present invention may be used to produce ligands which are designed to kill or inhibit any specific target organism(s).

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

INCL INCLM: 514/002.000

INCLS: 702/019.000; 702/027.000; 530/350.000

NCL NCLM: 514/002.000

NCLS: 702/019.000; 702/027.000; 530/350.000

L15 ANSWER 2 OF 23 USPATFULL on STN

ACCESSION NUMBER: 2003:258355 USPATFULL

TITLE: Genes essential for microbial proliferation and antisense thereto

INVENTOR(S): Forsyth, R. Allyn, San Diego, CA, UNITED STATES  
Ohlsen, Kari, San Diego, CA, UNITED STATES  
Zyskind, Judith W., La Jolla, CA, UNITED STATES

NUMBER	KIND	DATE
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Searcher : Shears 308-4994

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PATENT INFORMATION: US 2003181408 A1 20030925  
APPLICATION INFO.: US 2002-287274 A1 20021031 (10)  
RELATED APPLN. INFO.: Division of Ser. No. US 2000-711164, filed on 9 Nov 2000, GRANTED, Pat. No. US 6589738

	NUMBER	DATE
PRIORITY INFORMATION:	US 1999-164415P	19991109 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	APPLICATION	
LEGAL REPRESENTATIVE:	KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614	
NUMBER OF CLAIMS:	68	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	3 Drawing Page(s)	
LINE COUNT:	4685	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The sequences of nucleic acids encoding proteins required for E. coli proliferation are disclosed. The nucleic acids can be used to express proteins or portions thereof, to obtain antibodies capable of specifically binding to the expressed proteins, and to use those expressed proteins as a screen to isolate candidate molecules for rational drug discovery programs. The nucleic acids can also be used to screen for homologous genes that are required for proliferation in microorganisms other than E. Coli. The nucleic acids can also be used to design expression vectors and secretion vectors. The nucleic acids of the present invention can also be used in various assay systems to screen for proliferation required genes in other organisms as well as to screen for antimicrobial agents.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

INCL INCLM: 514/044.000  
INCLS: 435/375.000; 435/456.000  
NCL NCLM: 514/044.000  
NCLS: 435/375.000; 435/456.000

L15 ANSWER 3 OF 23 USPATFULL on STN  
ACCESSION NUMBER: 2003:250914 USPATFULL  
TITLE: Compounds and methods for treatment and diagnosis of chlamydial infection  
INVENTOR(S): Bhatia, Ajay, Seattle, WA, UNITED STATES  
                  Probst, Peter, Seattle, WA, UNITED STATES  
                  Stromberg, Erika Jean, Seattle, WA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003175700	A1	20030918
APPLICATION INFO.:	US 2001-841260	A1	20010423 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-198853P	20000421 (60)
	US 2000-219752P	20000720 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	APPLICATION	
LEGAL REPRESENTATIVE:	SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701	

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FIFTH AVE, SUITE 6300, SEATTLE, WA, 98104-7092

NUMBER OF CLAIMS: 18

EXEMPLARY CLAIM: 1

LINE COUNT: 9573

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Compounds and methods for the diagnosis and treatment of Chlamydial infection are disclosed. The compounds provided include polypeptides that contain at least one antigenic portion of a Chlamydia antigen and DNA sequences encoding such polypeptides. Pharmaceutical compositions and vaccines comprising such polypeptides or DNA sequences are also provided, together with antibodies directed against such polypeptides. Diagnostic kits containing such polypeptides or DNA sequences and a suitable detection reagent may be used for the detection of Chlamydial infection in patients and in biological samples.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

INCL INCLM: 435/006.000

INCLS: 435/007.360; 435/069.300; 435/252.300; 435/320.100;  
435/183.000; 536/023.700; 530/350.000; 424/190.100

NCL NCLM: 435/006.000

NCLS: 435/007.360; 435/069.300; 435/252.300; 435/320.100;  
435/183.000; 536/023.700; 530/350.000; 424/190.100

L15 ANSWER 4 OF 23 USPATFULL on STN

ACCESSION NUMBER: 2003:244913 USPATFULL

TITLE: Determination and uses of the atomic structures of ribosomes and ribosomal subunits and their ligand complexes

INVENTOR(S): Steitz, Thomas A., Branford, CT, UNITED STATES  
Moore, Peter B., North Haven, CT, UNITED STATES  
Ban, Nenad, Zurich, SWITZERLAND  
Nissen, Poul, Aarhus N, DENMARK  
Hansen, Jeffrey, New Haven, CT, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003171327	A1	20030911
APPLICATION INFO.:	US 2003-391491	A1	20030317 (10)
RELATED APPLN. INFO.:	Continuation of Ser. No. US 2000-635708, filed on 9 Aug 2000, PENDING		

DOCUMENT TYPE: Utility

FILE SEGMENT: APPLICATION

LEGAL REPRESENTATIVE: TESTA, HURWITZ & THIBEAULT, LLP, HIGH STREET  
TOWER, 125 HIGH STREET, BOSTON, MA, 02110

NUMBER OF CLAIMS: 40

EXEMPLARY CLAIM: 1

NUMBER OF DRAWINGS: 22 Drawing Page(s)

LINE COUNT: 4037

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides methods for producing high resolution crystals of ribosomes and ribosomal subunits as well as the crystals produced by such methods. The x-ray diffraction patterns of the crystals provided by the present invention are of sufficiently high resolution for determining the three-dimensional structure of ribosomes and ribosomal subunits, for identifying ligand binding sites on ribosomes and ribosomal subunits, and for molecular modeling of ligands which interact with ribosomes and

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ribosomal subunits. The present invention provides methods for identifying ribosome-related ligands and methods for designing ligands with specific ribosome-binding properties. Thus, the methods of the present invention may be used to produce ligands which are designed to kill or inhibit any specific target organism(s).

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

INCL INCLM: 514/044.000  
INCLS: 702/020.000; 536/023.100  
NCL NCLM: 514/044.000  
NCLS: 702/020.000; 536/023.100

L15 ANSWER 5 OF 23 USPATFULL on STN

ACCESSION NUMBER: 2003:244285 USPATFULL  
TITLE: Stabilized nucleic acids in gene and drug discovery and methods of use  
INVENTOR(S): Wall, Daniel, San Diego, CA, UNITED STATES  
Froelich, Jamie, San Diego, CA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003170694	A1	20030911
APPLICATION INFO.:	US 2002-327592	A1	20021220 (10)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2001-343512P	20011221 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	APPLICATION	
LEGAL REPRESENTATIVE:	KNOBBE MARTENS OLSON & BEAR LLP, 2040 MAIN STREET, FOURTEENTH FLOOR, IRVINE, CA, 92614	
NUMBER OF CLAIMS:	37	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	8 Drawing Page(s)	
LINE COUNT:	5963	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Stabilized nucleic acids for use in gene and drug discovery are disclosed. Vectors and host cells useful in the production of stabilized nucleic acids are also disclosed. Cell-based assays which employ stabilized antisense nucleic acids to identify and develop antibiotics and to identify genes required for proliferation are described. The use of stabilized nucleic acids to identify homologous nucleic acids required for the proliferation of heterologous organisms is also described. Inhibition of the expression of genes required for proliferation in heterologous organisms through the use of stabilized antisense nucleic acids is disclosed.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

INCL INCLM: 435/006.000  
INCLS: 435/032.000  
NCL NCLM: 435/006.000  
NCLS: 435/032.000

L15 ANSWER 6 OF 23 USPATFULL on STN

ACCESSION NUMBER: 2003:244254 USPATFULL  
TITLE: Nucleotide sequence of the Mycoplasma genitalium

Searcher : Shears 308-4994

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INVENTOR(S): genome, fragments thereof, and uses thereof  
Fraser, Claire M., Potomac, MD, UNITED STATES  
Adams, Mark D., Rockville, MD, UNITED STATES  
Gocayne, Jeannine D., Potomac, MD, UNITED STATES  
Hutchison, Clyde A., III, Chapel Hill, MD, UNITED  
STATES  
Smith, Hamilton O., Reisterstown, MD, UNITED  
STATES  
Venter, J. Craig, Queenstown, MD, UNITED STATES  
White, Owen R., Rockville, MD, UNITED STATES  
Johns Hopkins University, Baltimore, MD (U.S.  
corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003170663	A1	20030911
APPLICATION INFO.:	US 2002-205220	A1	20020726 (10)
RELATED APPLN. INFO.:	Division of Ser. No. US 1995-545528, filed on 19 Oct 1995, PENDING Continuation-in-part of Ser. No. US 1995-488018, filed on 7 Jun 1995, PENDING Continuation-in-part of Ser. No. US 1995-473545, filed on 7 Jun 1995, ABANDONED		

DOCUMENT TYPE: Utility  
FILE SEGMENT: APPLICATION  
LEGAL REPRESENTATIVE: HUMAN GENOME SCIENCES INC, 9410 KEY WEST AVENUE,  
ROCKVILLE, MD, 20850  
NUMBER OF CLAIMS: 19  
EXEMPLARY CLAIM: 1  
NUMBER OF DRAWINGS: 23 Drawing Page(s)  
LINE COUNT: 6270

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides the nucleotide sequence of the entire genome of Mycoplasma genitalium, SEQ ID NO: 1. The present invention further provides the sequence information stored on computer readable media, and computer-based systems and methods which facilitate its use. In addition to the entire genomic sequence, the present invention identifies protein encoding fragments of the genome, and identifies, by position relative to two (2) genes known to flank the origin of replication, any regulatory elements which modulate the expression of the protein encoding fragments of the Mycoplasma genitalium genome.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

INCL INCLM: 435/006.000  
INCLS: 435/069.100; 435/183.000; 435/252.300; 435/320.100;  
536/023.700  
NCL NCLM: 435/006.000  
NCLS: 435/069.100; 435/183.000; 435/252.300; 435/320.100;  
536/023.700

L15 ANSWER 7 OF 23 USPATFULL on STN

ACCESSION NUMBER: 2003:240330 USPATFULL  
TITLE: Nucleic acid and amino acid sequences relating to  
Enterococcus faecalis for diagnostics and  
therapeutics  
INVENTOR(S): Doucette-Stamm, Lynn A., 14 Flanagan Dr.,  
Framingham, MA, United States 01701  
Bush, David, 205 Holland St., Somerville, MA,

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United States 02144

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6617156	B1	20030909
APPLICATION INFO.:	US 1998-134000		19980813 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 1997-55778P	19970815 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	GRANTED	
PRIMARY EXAMINER:	Mosher, Mary E.	
LEGAL REPRESENTATIVE:	Genome Therapeutics Corporation	
NUMBER OF CLAIMS:	19	
EXEMPLARY CLAIM:	1,5,14	
NUMBER OF DRAWINGS:	0 Drawing Figure(s); 0 Drawing Page(s)	
LINE COUNT:	13738	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides isolated polypeptide and nucleic acid sequences derived from Enterococcus faecalis that are useful in diagnosis and therapy of pathological conditions; antibodies against the polypeptides; and methods for the production of the polypeptides. The invention also provides methods for the detection, prevention and treatment of pathological conditions resulting from bacterial infection.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

INCL INCLM: 435/320.100  
INCLS: 536/023.700; 536/024.320; 435/252.300; 435/069.100;  
435/006.000  
NCL NCLM: 435/320.100  
NCLS: 536/023.700; 536/024.320; 435/252.300; 435/069.100;  
435/006.000

L15 ANSWER 8 OF 23 USPATFULL on STN

ACCESSION NUMBER: 2003:219712 USPATFULL  
TITLE: Ribosome structure and protein synthesis inhibitors  
INVENTOR(S): Steitz, Thomas A., Branford, CT, UNITED STATES  
Moore, Peter B., North Haven, CT, UNITED STATES  
Ban, Nenad, New Haven, CT, UNITED STATES  
Nissen, Poul, Aarhus N, DENMARK  
Hansen, Jeffrey, New Haven, CT, UNITED STATES  
Ippolito, Joseph A., Guilford, CT, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003153002	A1	20030814
APPLICATION INFO.:	US 2002-72634	A1	20020208 (10)
RELATED APPLN. INFO.:	Continuation of Ser. No. US 2001-922251, filed on 3 Aug 2001, PENDING		

	NUMBER	DATE
PRIORITY INFORMATION:	US 2002-348731P	20020114 (60)
	US 2002-352024P	20020125 (60)
DOCUMENT TYPE:	Utility	

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FILE SEGMENT: APPLICATION  
LEGAL REPRESENTATIVE: TESTA, HURWITZ & THIBEAULT, LLP, HIGH STREET  
TOWER, 125 HIGH STREET, BOSTON, MA, 02110  
NUMBER OF CLAIMS: 106  
EXEMPLARY CLAIM: 1  
NUMBER OF DRAWINGS: 36 Drawing Page(s)  
LINE COUNT: 8432  
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides methods for producing high resolution crystals of ribosomes and ribosomal subunits as well as crystals produced by such methods. The invention also provides high resolution structures of ribosomal subunits either alone or in combination with protein synthesis inhibitors. The invention provides methods for identifying ribosome-related ligands and methods for designing ligands with specific ribosome-binding properties as well as ligands that may act as protein synthesis inhibitors. Thus, the methods and compositions of the invention may be used to produce ligands that are designed to specifically kill or inhibit the growth of any target organism.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

INCL INCLM: 435/007.100  
INCLS: 702/019.000  
NCL NCLM: 435/007.100  
NCLS: 702/019.000

L15 ANSWER 9 OF 23 USPATFULL on STN  
ACCESSION NUMBER: 2003:194596 USPATFULL  
TITLE: Nucleic acids and proteins from Streptococcus pneumoniae  
INVENTOR(S): Le Page, Richard William Falla, London, UNITED KINGDOM  
Wells, Jeremy Mark, Norwich, UNITED KINGDOM  
Hanniffy, Sean Bosco, Cambridge, UNITED KINGDOM  
Hansbro, Philip Michael, Newcastle, AUSTRALIA

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003134407	A1	20030717
APPLICATION INFO.:	US 2001-769744	A1	20010126 (9)
RELATED APPLN. INFO.:	Continuation of Ser. No. WO 1999-GB2452, filed on 27 Jul 1999, UNKNOWN		

	NUMBER	DATE
PRIORITY INFORMATION:	GB 1998-16336	19980727
	US 1999-125329P	19990319 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	APPLICATION	
LEGAL REPRESENTATIVE:	BROBECK, PHLEGER & HARRISON, LLP, ATTN: INTELLECTUAL PROPERTY DEPARTMENT, 1333 H STREET, N.W. SUITE 800, WASHINGTON, DC, 20005	
NUMBER OF CLAIMS:	20	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	2 Drawing Page(s)	
LINE COUNT:	9110	
CAS INDEXING IS AVAILABLE FOR THIS PATENT.		
AB	Novel proteins from Streptococcus pneumoniae are described,	

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together with nucleic acid sequences encoding them. Their use in vaccines and in screening methods is also described.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

INCL INCLM: 435/252.300  
INCLS: 530/350.000; 536/023.100  
NCL NCLM: 435/252.300  
NCLS: 530/350.000; 536/023.100

L15 ANSWER 10 OF 23 USPATFULL on STN

ACCESSION NUMBER: 2003:190673 USPATFULL

TITLE: Staphylococcus aureus polynucleotides and sequences

INVENTOR(S): Kunsch, Charles A., Norcross, GA, United States  
Choi, Gil H., Rockville, MD, United States  
Barash, Steven, Rockville, MD, United States  
Dillon, Patrick J., Carlsbad, CA, United States  
Fannon, Michael R., Silver Spring, MD, United States

PATENT ASSIGNEE(S): Rosen, Craig A., Laytonsville, MD, United States  
Human Genome Sciences, Inc., Rockville, MD,  
United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6593114	B1	20030715
APPLICATION INFO.:	US 1997-956171		19971020 (8)
RELATED APPLN. INFO.:	Continuation-in-part of Ser. No. US 1997-781986, filed on 3 Jan 1997		

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-9861P	19960105 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	GRANTED	
PRIMARY EXAMINER:	Duffy, Patricia A.	
LEGAL REPRESENTATIVE:	Human Genome Sciences, Inc.	
NUMBER OF CLAIMS:	15	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	2 Drawing Figure(s); 2 Drawing Page(s)	
LINE COUNT:	7835	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides polynucleotide sequences of the genome of Staphylococcus aureus, polypeptide sequences encoded by the polynucleotide sequences, corresponding polynucleotides and polypeptides, vectors and hosts comprising the polynucleotides, and assays and other uses thereof. The present invention further provides polynucleotide and polypeptide sequence information stored on computer readable media, and computer-based systems and methods which facilitate its use.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

INCL INCLM: 435/091.410  
INCLS: 435/091.400; 435/252.300; 435/254.110; 435/257.200;  
435/320.100; 435/325.000; 536/023.700  
NCL NCLM: 435/091.410  
NCLS: 435/091.400; 435/252.300; 435/254.110; 435/257.200;  
435/320.100; 435/325.000; 536/023.700

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L15 ANSWER 11 OF 23 USPATFULL on STN  
ACCESSION NUMBER: 2003:183969 USPATFULL  
TITLE: Genes essential for microbial proliferation and antisense thereto  
INVENTOR(S): Forsyth, R. Allyn, San Diego, CA, United States  
Ohlsen, Kari, San Diego, CA, United States  
Zyskind, Judith W., La Jolla, CA, United States  
PATENT ASSIGNEE(S): Elitra Pharmaceuticals, Inc., San Diego, CA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6589738	B1	20030708
APPLICATION INFO.:	US 2000-711164		20001109 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 1999-164415P	19991109 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	GRANTED	
PRIMARY EXAMINER:	Jones, W. Gary	
ASSISTANT EXAMINER:	Taylor, Janell E.	
LEGAL REPRESENTATIVE:	Knobbe, Martens, Olson & Bear LLP	
NUMBER OF CLAIMS:	12	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	4 Drawing Figure(s); 3 Drawing Page(s)	
LINE COUNT:	4292	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The sequences of nucleic acids encoding proteins required for E. Coli proliferation are disclosed. The nucleic acids can be used to express proteins or portions thereof, to obtain antibodies capable of specifically binding to the expressed proteins, and to use those expressed proteins as a screen to isolate candidate molecules for rational drug discovery programs. The nucleic acids can also be used to screen for homologous genes that are required for proliferation in microorganisms other than E. Coli. The nucleic acids can also be used to design expression vectors and secretion vectors. The nucleic acids of the present invention can also be used in various assay systems to screen for proliferation required genes in other organisms as well as to screen for antimicrobial agents.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

INCL INCLM: 435/006.000  
INCLS: 435/005.000; 435/091.100; 435/091.200; 536/023.100;  
536/024.300; 536/024.500; 536/024.310; 536/024.330;  
530/350.000  
NCL NCLM: 435/006.000  
NCLS: 435/005.000; 435/091.100; 435/091.200; 530/350.000;  
536/023.100; 536/024.300; 536/024.310; 536/024.330;  
536/024.500

L15 ANSWER 12 OF 23 USPATFULL on STN  
ACCESSION NUMBER: 2003:133492 USPATFULL  
TITLE: Streptococcus pneumoniae proteins and nucleic acid molecules  
INVENTOR(S): Gilbert, Christophe Francois Guy, Villeurbanne

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cedex, FRANCE  
Hansbro, Philip Michael, Newcastle, AUSTRALIA

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003091577	A1	20030515
APPLICATION INFO.:	US 2001-769787	A1	20010126 (9)
RELATED APPLN. INFO.:	Continuation of Ser. No. WO 1999-GB2451, filed on 27 Jul 1999, UNKNOWN		

	NUMBER	DATE
PRIORITY INFORMATION:	DE 1998-16337	19980727
	US 1999-125164P	19990319 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	APPLICATION	
LEGAL REPRESENTATIVE:	BROBECK, PHLEGER & HARRISON, LLP, ATTN: INTELLECTUAL PROPERTY DEPARTMENT, 1333 H STREET, N.W. SUITE 800, WASHINGTON, DC, 20005	
NUMBER OF CLAIMS:	20	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	2 Drawing Page(s)	
LINE COUNT:	4964	
CAS INDEXING IS AVAILABLE FOR THIS PATENT.		

AB Novel protein antigens from *Streptococcus pneumoniae* are disclosed, together with nucleic acid sequences encoding them. Their use in vaccines and in screening methods is also described.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

INCL INCLM: 424/184.100  
INCLS: 530/350.000  
NCL NCLM: 424/184.100  
NCLS: 530/350.000

L15 ANSWER 13 OF 23 USPATFULL on STN  
ACCESSION NUMBER: 2003:130010 USPATFULL  
TITLE: Nucleic acid and amino acid sequences relating to *Acinetobacter baumannii* for diagnostics and therapeutics  
INVENTOR(S): Breton, Gary, Marlborough, MA, United States  
Bush, David, Somerville, MA, United States  
PATENT ASSIGNEE(S): Genome Therapeutics Corporation, Waltham, MA, United States (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6562958	B1	20030513
APPLICATION INFO.:	US 1999-328352		19990604 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 1998-88701P	19980609 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	GRANTED	
PRIMARY EXAMINER:	Borin, Michael	
LEGAL REPRESENTATIVE:	Genome Therapeutics Corporation	
NUMBER OF CLAIMS:	15	
EXEMPLARY CLAIM:	1	

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NUMBER OF DRAWINGS: 0 Drawing Figure(s); 0 Drawing Page(s)  
LINE COUNT: 16618

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides isolated polypeptide and nucleic acid sequences derived from *Acinetobacter mirabilis* that are useful in diagnosis and therapy of pathological conditions; antibodies against the polypeptides; and methods for the production of the polypeptides. The invention also provides methods for the detection, prevention and treatment of pathological conditions resulting from bacterial infection.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

INCL INCLM: 536/023.700

INCLS: 536/023.100

NCL NCLM: 536/023.700

NCLS: 536/023.100

L15 ANSWER 14 OF 23 USPATFULL on STN

ACCESSION NUMBER: 2003:81597 USPATFULL

TITLE: Nucleotide sequence of the mycoplasma genitalium genome, fragments thereof, and uses thereof  
Fraser, Claire M., Potomac, MD, United States  
Adams, Mark D., N. Potomac, MD, United States  
Gocayne, Jeannine D., Silver Spring, MD, United States  
Hutchison, III, Clyde A., Chapel Hill, NC, United States  
Smith, Hamilton O., Towson, MD, United States  
Venter, J. Craig, Potomac, MD, United States  
White, Owen, Gaithersburg, MD, United States

INVENTOR(S):  
PATENT ASSIGNEE(S):  
The Institute for Genomic Research, Rockville, MD, United States (U.S. corporation)  
Johns Hopkins University, Baltimore, MD, United States (U.S. corporation)  
The University of North Carolina at Chapel Hill, Chapel Hill, NC, United States (U.S. corporation)

NUMBER	KIND	DATE
US 6537773	B1	20030325
US 1995-545528		19951019 (8)
Continuation-in-part of Ser. No. US 1995-488018, filed on 7 Jun 1995, now abandoned		
Continuation-in-part of Ser. No. US 1995-473545, filed on 7 Jun 1995, now abandoned		

DOCUMENT TYPE: Utility

FILE SEGMENT: GRANTED

PRIMARY EXAMINER: Ketter, James

ASSISTANT EXAMINER: Schnizer, Richard

LEGAL REPRESENTATIVE: Human Genome Sciences, Inc.

NUMBER OF CLAIMS: 44

EXEMPLARY CLAIM: 1

NUMBER OF DRAWINGS: 23 Drawing Figure(s); 23 Drawing Page(s)

LINE COUNT: 15190

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides the nucleotide sequence of the entire genome of *Mycoplasma genitalium*, SEQ ID NO:1. The present invention further provides the sequence information stored on

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computer readable media, and computer-based systems and methods which facilitate its use. In addition to the entire genomic sequence, the present invention identifies protein encoding fragments of the genome, and identifies, by position relative to two (2) genes known to flank the origin of replication, any regulatory elements which modulate the expression of the protein encoding fragments of the *Mycoplasma genitalium* genome.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

INCL INCLM: 435/069.100  
INCLS: 536/023.700; 536/024.320; 435/252.300; 435/320.100  
NCL NCLM: 435/069.100  
NCLS: 435/252.300; 435/320.100; 536/023.700; 536/024.320

L15 ANSWER 15 OF 23 USPATFULL on STN

ACCESSION NUMBER: 2003:78516 USPATFULL

TITLE: STAPHYLOCOCCUS AUREUS POLYNUCLEOTIDES AND  
SEQUENCES

INVENTOR(S): KUNSCH, CHARLES A., GAITHERSBURG, MD, UNITED  
STATES  
CHOI, GIL A., ROCKVILLE, MD, UNITED STATES  
BARASH, STEVEN C., ROCKVILLE, MD, UNITED STATES  
DILLON, PATRICK J., GAITHERSBURG, MD, UNITED  
STATES  
FANNON, MICHAEL R., SILVER SPRING, MD, UNITED  
STATES  
ROSEN, CRAIG A., LAYTONSVILLE, MD, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003054436	A1	20030320
APPLICATION INFO.:	US 1997-781986	A1	19970103 (8)

	NUMBER	DATE
PRIORITY INFORMATION:	US 1996-9861P	19960105 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	APPLICATION	
LEGAL REPRESENTATIVE:	HUMAN GENOME SCIENCES INC, 9410 KEY WEST AVENUE, ROCKVILLE, MD, 20850	
NUMBER OF CLAIMS:	29	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	2 Drawing Page(s)	
LINE COUNT:	13414	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The present invention provides polynucleotide sequences of the genome of *Staphylococcus aureus*, polypeptide sequences encoded by the polynucleotide sequences, corresponding polynucleotides and polypeptides, vectors and hosts comprising the polynucleotides, and assays and other uses thereof. The present invention further provides polynucleotide and polypeptide sequence information stored on computer readable media, and computer-based systems and methods which facilitate its use.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

INCL INCLM: 435/069.100  
INCLS: 536/023.700; 536/023.100; 435/320.100; 435/252.300  
NCL NCLM: 435/069.100

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NCLS: 536/023.700; 536/023.100; 435/320.100; 435/252.300

L15 ANSWER 16 OF 23 USPATFULL on STN  
ACCESSION NUMBER: 2003:37641 USPATFULL  
TITLE: Bacterial promoters and methods of use  
INVENTOR(S): Haselbeck, Robert, San Diego, CA, UNITED STATES  
Wall, Daniel, San Diego, CA, UNITED STATES  
Gross, Molly, San Diego, CA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2003027286	A1	20030206
APPLICATION INFO.:	US 2001-32393	A1	20011221 (10)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-259434P	20001227 (60)
	US 2000-230335P	20000906 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	APPLICATION	
LEGAL REPRESENTATIVE:	KNOBBE MARTENS OLSON & BEAR LLP, 620 NEWPORT CENTER DRIVE, SIXTEENTH FLOOR, NEWPORT BEACH, CA, 92660	
NUMBER OF CLAIMS:	135	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	17 Drawing Page(s)	
LINE COUNT:	9146	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Compositions and methods are disclosed herein that relate to the development of fusion promoters for regulating gene expression in bacteria. Embodiments include fusion promoters comprising one or more operators linked to a promoter that is modified to have altered activity in Gram-positive organisms. Vectors and cells containing these fusion promoters are also described. Other embodiments include, methods of using these fusion promoters to regulate nucleic acid and/or polypeptide expression, methods of using these fusion promoters to identify proliferation-required genes, and methods of using these fusion promoters to identify molecules having potential antibiotic activity.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

INCL INCLM: 435/069.600  
INCLS: 435/219.000; 435/320.100; 435/252.300; 536/023.200;  
435/006.000  
NCL NCLM: 435/069.600  
NCLS: 435/219.000; 435/320.100; 435/252.300; 536/023.200;  
435/006.000

L15 ANSWER 17 OF 23 USPATFULL on STN  
ACCESSION NUMBER: 2003:26250 USPATFULL  
TITLE: Elongation factor P (EFP) and assays and antimicrobial treatments related to the same  
INVENTOR(S): Marotti, Keith R., Kalamazoo, MI, United States  
Poorman, Roger A., Kalamazoo, MI, United States  
Wells, Peter A., Kalamazoo, MI, United States  
Shinabarger, Dean L., Portage, MI, United States  
PATENT ASSIGNEE(S): Pharmacia & Upjohn Company, Kalamazoo, MI, United States (U.S. corporation)

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	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6511813	B1	20030128
APPLICATION INFO.:	US 2000-704321		20001102 (9)
RELATED APPLN. INFO.:	Division of Ser. No. US 1999-322732, filed on 28 May 1999		

	NUMBER	DATE
PRIORITY INFORMATION:	US 1999-117473P	19990127 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	GRANTED	
PRIMARY EXAMINER:	Cochrane Carlson, Karen	
ASSISTANT EXAMINER:	Robinson, Hope A.	
LEGAL REPRESENTATIVE:	O'Connor, P.C., Cozen	
NUMBER OF CLAIMS:	9	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	0 Drawing Figure(s); 0 Drawing Page(s)	
LINE COUNT:	1234	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Disclosed are novel methods of using elongation factor p (efp) and related constituents of ribosomal complexes which comprise efp, the 50S ribosomal subunit, the 30S ribosomal subunit, the 70S initiation complex, and related proteins, cofactors and enzymes. Methods of identifying compounds which modulate prokaryotic elongation factor p and modify cell function are described. Both in vitro and in vivo methods for identifying compounds which modulate such constituents and affect cell function are described. Such identified compounds, including various antibiotics, which specifically affect cell growth, methods of treating various disorders with such compounds, and antiseptics containing such compounds are described. The present invention is also directed to methods and compounds that modulate prokaryotic elongation factor p.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

INCL INCLM: 435/007.100  
INCLS: 435/006.000; 530/350.000; 530/300.000; 536/023.100;  
514/002.000  
NCL NCLM: 435/007.100  
NCLS: 435/006.000; 514/002.000; 530/300.000; 530/350.000;  
536/023.100

L15 ANSWER 18 OF 23 USPATFULL on STN  
ACCESSION NUMBER: 2002:343879 USPATFULL  
TITLE: Novel Polynucleotides  
INVENTOR(S): Nakagawa, Satoshi, Tokyo, JAPAN  
Mizoguchi, Hiroshi, Tokyo, JAPAN  
Ando, Seiko, Tokyo, JAPAN  
Hayashi, Mikiro, Tokyo, JAPAN  
Ochiai, Keiko, Tokyo, JAPAN  
Yokoi, Haruhiko, Tokyo, JAPAN  
Tateishi, Naoko, Tokyo, JAPAN  
Senoh, Akihiro, Tokyo, JAPAN  
Ikeda, Masato, Tokyo, JAPAN  
Ozaki, Akio, Hofu-shi, JAPAN

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	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002197605	A1	20021226
APPLICATION INFO.:	US 2000-738626	A1	20001218 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	JP 1999-377484	19991216
	JP 2000-159162	20000407
	JP 2000-280988	20000803

DOCUMENT TYPE:

FILE SEGMENT:

LEGAL REPRESENTATIVE: NIXON & VANDERHYE P.C., 8th Floor, 1100 North Glebe Road, Arlington, VA, 22201

NUMBER OF CLAIMS: 68

EXEMPLARY CLAIM: 1

NUMBER OF DRAWINGS: 4 Drawing Page(s)

LINE COUNT: 13673

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Novel polynucleotides derived from microorganisms belonging to coryneform bacteria and fragments thereof, polypeptides encoded by the polynucleotides and fragments thereof, polynucleotide arrays comprising the polynucleotides and fragments thereof, recording media in which the nucleotide sequences of the polynucleotide and fragments thereof have been recorded which are readable in a computer, and use of them.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

INCL INCLM: 435/006.000

INCLS: 435/091.200; 435/287.200

NCL NCLM: 435/006.000

NCLS: 435/091.200; 435/287.200

L15 ANSWER 19 OF 23 USPATFULL on STN

ACCESSION NUMBER: 2002:265900 USPATFULL

TITLE: Compounds and methods for treatment and diagnosis of chlamydial infection

INVENTOR(S): Bhatia, Ajay, Seattle, WA, UNITED STATES

Probst, Peter, Seattle, WA, UNITED STATES

PATENT ASSIGNEE(S): Corixa Corporation, Seattle, WA, UNITED STATES, 98104 (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002146776	A1	20021010
APPLICATION INFO.:	US 2001-7693	A1	20011205 (10)
RELATED APPLN. INFO.:	Continuation-in-part of Ser. No. US 2001-841260, filed on 23 Apr 2001, PENDING		

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-219752P	20000720 (60)
	US 2000-198853P	20000421 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	APPLICATION	
LEGAL REPRESENTATIVE:	SEED INTELLECTUAL PROPERTY LAW GROUP PLLC, 701 FIFTH AVE, SUITE 6300, SEATTLE, WA, 98104-7092	
NUMBER OF CLAIMS:	17	

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EXEMPLARY CLAIM: 1  
LINE COUNT: 4342

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB Compounds and methods for the diagnosis and treatment of Chlamydial infection are disclosed. The compounds provided include polypeptides that contain at least one antigenic portion of a Chlamydia antigen and DNA sequences encoding such polypeptides. Pharmaceutical compositions and vaccines comprising such polypeptides or DNA sequences are also provided, together with antibodies directed against such polypeptides. Diagnostic kits containing such polypeptides or DNA sequences and a suitable detection reagent may be used for the detection of Chlamydial infection in patients and in biological samples.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

INCL INCLM: 435/069.300  
INCLS: 435/252.300; 435/320.100; 435/183.000; 536/023.700  
NCL NCLM: 435/069.300  
NCLS: 435/252.300; 435/320.100; 435/183.000; 536/023.700

L15 ANSWER 20 OF 23 USPATFULL on STN

ACCESSION NUMBER: 2002:164690 USPATFULL

TITLE: Ribosome structure and protein synthesis inhibitors

INVENTOR(S): Steitz, Thomas A., Branford, CT, UNITED STATES  
Moore, Peter B., New Haven, CT, UNITED STATES  
Ban, Nenad, Riedenhalden, SWITZERLAND  
Nissen, Poul, Aarhus N, DENMARK  
Hansen, Jeffrey, New Haven, CT, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002086308	A1	20020704
APPLICATION INFO.:	US 2001-922251	A1	20010803 (9)
RELATED APPLN. INFO.:	Continuation-in-part of Ser. No. US 2000-653708, filed on 1 Sep 2000, GRANTED, Pat. No. US 6265725		

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-223977P	20000809 (60)
	US 2001-306996P	20010720 (60)
	US 309281P	(60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	APPLICATION	
LEGAL REPRESENTATIVE:	MORGAN LEWIS & BOCKIUS LLP, 1111 PENNSYLVANIA AVENUE, N.W., WASHINGTON, DC, 20004	
NUMBER OF CLAIMS:	112	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	30 Drawing Page(s)	
LINE COUNT:	6385	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention provides methods for producing high resolution crystals of ribosomes and ribosomal subunits as well as crystals produced by such methods. The invention also provides high resolution structures of ribosomal subunits either alone or in combination with protein synthesis inhibitors. The invention provides methods for identifying ribosome-related ligands and methods for designing ligands with specific ribosome-binding

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properties as well as ligands that may act as protein synthesis inhibitors. Thus, the methods and compositions of the invention may be used to produce ligands that are designed to specifically kill or inhibit the growth of any target organism.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

INCL INCLM: 435/006.000  
INCLS: 702/019.000; 378/073.000  
NCL NCLM: 435/006.000  
NCLS: 702/019.000; 378/073.000

L15 ANSWER 21 OF 23 USPATFULL on STN

ACCESSION NUMBER: 2002:119586 USPATFULL  
TITLE: Identification of essential genes in prokaryotes  
INVENTOR(S): Haselbeck, Robert, San Diego, CA, UNITED STATES  
Ohlsen, Kari L., San Diego, CA, UNITED STATES  
Zyskind, Judith W., La Jolla, CA, UNITED STATES  
Wall, Daniel, San Diego, CA, UNITED STATES  
Trawick, John D., La Mesa, CA, UNITED STATES  
Carr, Grant J., Escondido, CA, UNITED STATES  
Yamamoto, Robert T., San Diego, CA, UNITED STATES  
Xu, H. Howard, San Diego, CA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002061569	A1	20020523
APPLICATION INFO.:	US 2001-815242	A1	20010321 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 2000-191078P	20000321 (60)
	US 2000-206848P	20000523 (60)
	US 2000-207727P	20000526 (60)
	US 2000-242578P	20001023 (60)
	US 2000-253625P	20001127 (60)
	US 2000-257931P	20001222 (60)
	US 2001-269308P	20010216 (60)

DOCUMENT TYPE: Utility  
FILE SEGMENT: APPLICATION  
LEGAL REPRESENTATIVE: KNOBBE MARTENS OLSON & BEAR LLP, 620 NEWPORT CENTER DRIVE, SIXTEENTH FLOOR, NEWPORT BEACH, CA, 92660

NUMBER OF CLAIMS: 44  
EXEMPLARY CLAIM: 1  
NUMBER OF DRAWINGS: 4 Drawing Page(s)  
LINE COUNT: 30870

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The sequences of antisense nucleic acids which inhibit the proliferation of prokaryotes are disclosed. Cell-based assays which employ the antisense nucleic acids to identify and develop antibiotics are also disclosed. The antisense nucleic acids can also be used to identify proteins required for proliferation, express these proteins or portions thereof, obtain antibodies capable of specifically binding to the expressed proteins, and to use those expressed proteins as a screen to isolate candidate molecules for rational drug discovery programs. The nucleic acids can also be used to screen for homologous nucleic acids that are required for proliferation in cells other than *Staphylococcus*

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aureus, Salmonella typhimurium, Klebsiella pneumoniae, and Pseudomonas aeruginosa. The nucleic acids of the present invention can also be used in various assay systems to screen for proliferation required genes in other organisms.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

INCL INCLM: 435/183.000  
INCLS: 530/350.000; 435/006.000; 536/023.200; 435/069.100;  
435/320.100; 435/325.000  
NCL NCLM: 435/183.000  
NCLS: 530/350.000; 435/006.000; 536/023.200; 435/069.100;  
435/320.100; 435/325.000

L15 ANSWER 22 OF 23 USPATFULL on STN

ACCESSION NUMBER: 2002:85550 USPATFULL

TITLE: Genes identified as required for proliferation in escherichia coli

INVENTOR(S): Zyskind, Judith, La Jolla, CA, UNITED STATES  
Ohlsen, Kari L., San Diego, CA, UNITED STATES  
Trawick, John, La Mesa, CA, UNITED STATES  
Forsyth, R. Allyn, San Diego, CA, UNITED STATES  
Froelich, Jamie M., San Diego, CA, UNITED STATES  
Carr, Grant J., Escondido, CA, UNITED STATES  
Yamamoto, Robert T., San Diego, CA, UNITED STATES  
Xu, H. Howard, San Diego, CA, UNITED STATES

PATENT ASSIGNEE(S): ELITRA PHARMACEUTICALS, INC. (U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002045592	A1	20020418
APPLICATION INFO.:	US 2001-912020	A1	20010723 (9)
RELATED APPLN. INFO.:	Division of Ser. No. US 2000-492709, filed on 27 Jan 2000, PENDING		

	NUMBER	DATE
PRIORITY INFORMATION:	US 1999-117405P	19990127 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	APPLICATION	
LEGAL REPRESENTATIVE:	KNOBBE MARTENS OLSON & BEAR LLP, 620 NEWPORT CENTER DRIVE, SIXTEENTH FLOOR, NEWPORT BEACH, CA, 92660	
NUMBER OF CLAIMS:	17	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	3 Drawing Page(s)	
LINE COUNT:	4246	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The sequences of nucleic acids encoding proteins required for E. coli proliferation are disclosed. The nucleic acids can be used to express proteins or portions thereof, to obtain antibodies capable of specifically binding to the expressed proteins, and to use those expressed proteins as a screen to isolate candidate molecules for rational drug discovery programs. The nucleic acids can also be used to screen for homologous genes that are required for proliferation in microorganisms other than E. coli. The nucleic acids can also be used to design expression vectors and secretion vectors. The nucleic acids of the present invention can also be used in various assay systems to screen for proliferation

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required genes in other organisms as well as to screen for antimicrobial agents.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

INCL INCLM: 514/044.000

INCLS: 435/476.000

NCL NCLM: 514/044.000

NCLS: 435/476.000

L15 ANSWER 23 OF 23 USPATFULL on STN

ACCESSION NUMBER: 2002:37998 USPATFULL

TITLE: Genes identified as required for proliferation of *E. coli*

INVENTOR(S): Forsyth, R. Allyn, San Diego, CA, UNITED STATES  
Ohlsen, Kari L., San Diego, CA, UNITED STATES  
Zyskind, Judith W., La Jolla, CA, UNITED STATES

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 2002022718	A1	20020221
APPLICATION INFO.:	US 2000-741669	A1	20001219 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 1999-173005P	19991223 (60)
DOCUMENT TYPE:	Utility	
FILE SEGMENT:	APPLICATION	
LEGAL REPRESENTATIVE:	KNOBBE MARTENS OLSON & BEAR LLP, 620 NEWPORT CENTER DRIVE, SIXTEENTH FLOOR, NEWPORT BEACH, CA, 92660	
NUMBER OF CLAIMS:	131	
EXEMPLARY CLAIM:	1	
NUMBER OF DRAWINGS:	3 Drawing Page(s)	
LINE COUNT:	5270	

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The sequences of nucleic acids encoding proteins required for *E. coli* proliferation are disclosed. The nucleic acids can also be used to screen for homologous genes that are required for proliferation in microorganisms other than *E. coli*. The nucleic acids can also be used to design expression vectors and secretion vectors. The nucleic acids can be used to express proteins or portions thereof, to obtain antibodies capable of specifically binding to the expressed proteins, and to use those expressed proteins as a screen to isolate candidate molecules for rational drug discovery programs. The nucleic acids of the present invention can also be used in various assay systems to screen for antimicrobial agents.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

INCL INCLM: 536/023.100

INCLS: 435/006.000; 435/069.100; 435/183.000; 435/325.000

NCL NCLM: 536/023.100

NCLS: 435/006.000; 435/069.100; 435/183.000; 435/325.000

(FILE 'HCAPLUS, MEDLINE, BIOSIS, EMBASE, WPIDS, CONFSCI, SCISEARCH, JICST-EPLUS, JAPIO, USPATFULL' ENTERED AT 14:46:22 ON 30 OCT 2003)

L16 450 S "RATTI G"?/AU

L17 55 S L16 AND L5

- Author

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L18 26 DUP REM L17 (29 DUPLICATES REMOVED)

L18 ANSWER 1 OF 26 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN  
ACCESSION NUMBER: 2003-532882 [50] WPIDS  
DOC. NO. CPI: C2003-144093  
TITLE: New immunogenic composition having a protein or  
encoding nucleic acid, useful for diagnosing,  
preventing and/or treating **Chlamydia**  
**trachomatis** infection.  
DERWENT CLASS: B04 D16  
INVENTOR(S): GRANDI, G; RATTI, G  
PATENT ASSIGNEE(S): (CHIR-N) CHIRON SPA  
COUNTRY COUNT: 102  
PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2003049762	A2	20030619	(200350)*	EN	163
RW:	AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SI SK SL SZ TR TZ UG ZM ZW				
W:	AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT RO RU SC SD SE SG SK SL TJ TM TN TR TT TZ UA UG US UZ VC VN YU ZA ZM ZW				

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2003049762	A2	WO 2002-IB5761	20021212

PRIORITY APPLN. INFO: GB 2002-18924 20020814; GB 2001-29732  
20011212; GB 2002-18233 20020806

AN 2003-532882 [50] WPIDS

AB WO2003049762 A UPAB: 20030805

NOVELTY - An immunogenic composition (I) comprising a protein or nucleic acid, and an adjuvant, where the protein or nucleic acid comprises any of 131 fully defined amino acid or nucleotide sequences given in the specification, or has 50% or greater sequence identity to it, or their fragments, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:

- (1) neutralizing **Chlamydia trachomatis** infectivity in a patient, comprising administering (I);
- (2) immunizing a patient against **C. trachomatis**, comprising administering (I);
- (3) raising antibodies specific for **C. trachomatis** elementary bodies, comprising administering (I);
- (4) raising antibodies which recognize the protein of (I), comprising administering a **C. trachomatis** elementary body; and
- (5) detecting a **C. trachomatis** elementary body in a biological sample, comprising contacting the sample with an antibody which recognizes a protein of (I).

ACTIVITY - Antibacterial.

In vitro neutralization assays for **Chlamydia**

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**trachomatis** were performed using LLCMK2 cells. The results showed that CT045, CT242, CT381, CT396, CT398, CT467, CT547, CT587 and CT681 were all particularly good candidates for vaccines to prevent infection by **C. trachomatis**.

MECHANISM OF ACTION - Vaccine; Gene-Therapy.

USE - The protein and/or nucleic acid of (I) is useful in the manufacture of a medicament for the treatment or prevention of infection due to **C. trachomatis**, where the infection is treated or prevented by the medicament eliciting an immune response which is specific to a **C. trachomatis** elementary body, or for neutralizing **C. trachomatis** elementary bodies (all claimed). They can also be used for the diagnosis of **C. trachomatis** infection.

Dwg. 0/44

L18 ANSWER 2 OF 26 HCPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 1

ACCESSION NUMBER: 2003:62220 HCPLUS

DOCUMENT NUMBER: 139:67455

TITLE: DNA immunization with pgp3 gene of  
**Chlamydia trachomatis** inhibits  
the spread of **chlamydial**  
infection from the lower to the upper  
genital tract in C3H/HeN mice

AUTHOR(S): Donati, Manuela; Sambri, Vittorio; Comanducci,  
Maurizio; Di Leo, Korinne; Storni, Elisa;  
Giacani, Lorenzo; Ratti, Giulio;  
Cevenini, Roberto

CORPORATE SOURCE: Ospedale Policlinico S. Orsola, Sezione di  
Microbiologia DMCSS, University of Bologna,  
Bologna, 40138, Italy

SOURCE: Vaccine (2003), 21(11-12), 1089-1093  
CODEN: VACCDE; ISSN: 0264-410X

PUBLISHER: Elsevier Science Ltd.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB **C. trachomatis** pgp3 DNA immunized and non-immunized C3H/HeN mice were infected by vaginal inoculation with infectious **C. trachomatis** serotype D elementary bodies (EBs) and the spread of infection to the salpinges was assessed by cell culture isolation from tissue homogenates 7, 14, 21, 28, 35 and 42 days post-infection (p.i.). Overall, the pgp3-DNA immunization prevented salpinx infection in 94 (56%) mice, if compared with the 168 pos. animals found among the non-immunized animals. A group of neg. control animals (i.e. mice immunized with plasmid DNA containing an irrelevant insert) was not protected, whereas all the mice of a pos. immune control group (mice that had resolved a primary genital **C. trachomatis** infection) were resistant to re-infection. Pgp3 DNA immunization induced both humoral and mucosal anti-pgp3 antibodies.

REFERENCE COUNT: 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 3 OF 26 HCPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 2

ACCESSION NUMBER: 2003:529165 HCPLUS

DOCUMENT NUMBER: 139:132194

TITLE: Mucosal and systemic immune responses to plasmid

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protein pgp3 in patients with genital and ocular  
**Chlamydia trachomatis**  
infection

AUTHOR(S): Ghaem-Maghami, S.; **Ratti, G.**; Ghaem-Maghami, M.; Comanducci, M.; Hay, P. E.; Bailey, R. L.; Mabey, D. C. W.; Whittle, H. C.; Ward, M. E.; Lewis, D. J. M.

CORPORATE SOURCE: Division of Infectious Diseases and Genito-Urinary Medicine, St. George's Hospital Medical School, UK

SOURCE: Clinical and Experimental Immunology (2003), 132(3), 436-442  
CODEN: CEXIAL; ISSN: 0009-9104

PUBLISHER: Blackwell Publishing Ltd.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The circulating and cervical B cell responses to **Chlamydia trachomatis** plasmid protein pgp3 were characterized in children and adults with ocular or genital **chlamydial infection** using the enzyme-linked immunospot assay (ELISPOT) and ELISA. No pgp3-specific ASCs were detected in healthy controls, but predominantly IgA ASCs were detected in UK adults with uncomplicated cervicitis or urethritis (0.019). In patients with extragenital complications or pelvic inflammatory disease a mixed response with more IgG and IgM ASCs was evident, suggesting a breach of mucosal immune compartmentalization with more extensive **infection**. In women with **chlamydial cervicitis**, ASCs secreting predominantly IgA, but also IgG, to pgp3 were present in cervix at presentation, with a frequency 30-50 times higher than blood. Cervical ASC nos., especially IgG, fell markedly six weeks after antibiotic treatment. The authors detected principally IgA pgp3-specific antibody secreting cells (ASCs) in children resident in a Gambian endemic area, with a trend towards suppression of IgA responses during intense **trachomatous** inflammation, as previously reported for other **chlamydial** antigens, and in keeping with the findings in genital disease. These data provide a rationale for further studies of immune responses to pgp3 in humans and animal models of **chlamydia**-induced disease, and its potential use in diagnostic assays and protective immunization strategies.

REFERENCE COUNT: 32 THERE ARE 32 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 4 OF 26 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN  
ACCESSION NUMBER: 2002-154726 [20] WPIDS  
DOC. NO. CPI: C2002-048393  
TITLE: Novel **Chlamydia pneumoniae** protein useful in the manufacture of a medicament for treatment or prevention of **infection** due to **Chlamydia**, preferably **Chlamydia pneumoniae**, and for diagnostic purposes.

DERWENT CLASS: B04 D16  
INVENTOR(S): GRANDI, G; **RATTI, G**  
PATENT ASSIGNEE(S): (CHIR-N) CHIRON SPA  
COUNTRY COUNT: 97  
PATENT INFORMATION:

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PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2002002606 A2	20020110 (200220)*	EN	364		
RW:	AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TR TZ UG ZW				
W:	AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW				
AU 2001076619 A	20020114 (200237)				
EP 1297005	A2 20030402 (200325)	EN			
R:	AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI TR				

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2002002606 A2		WO 2001-IB1445	20010703
AU 2001076619 A		AU 2001-76619	20010703
EP 1297005 A2		EP 2001-954278	20010703
		WO 2001-IB1445	20010703

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2001076619 A	Based on	WO 2002002606
EP 1297005 A2	Based on	WO 2002002606

PRIORITY APPLN. INFO: GB 2000-31706 20001222; GB 2000-16363  
20000703; GB 2000-17047 20000711; GB  
2000-17983 20000721; GB 2000-19368  
20000807; GB 2000-20440 20000818; GB  
2000-22583 20000914; GB 2000-27549 20001110

AN 2002-154726 [20] WPIDS  
AB WO 200202606 A UPAB: 20020402

NOVELTY - A **Chlamydia pneumoniae** protein (I) selected from a protein comprising one of 189 272-973 residue amino acid sequences (S1), all fully defined in the specification, a fragment of S1, or a protein having 50 % or greater sequence identity to S1, is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a nucleic acid molecule (II) selected from a molecule which encodes (I), a fragment of (II), a sequence complementary to them, a nucleic acid molecule comprising a sequence having 50 % or greater sequence identity to them, or a nucleic acid molecule which hybridizes to them under high stringency conditions; and

(2) a composition (III) comprising (I) or (II).

ACTIVITY - Antibacterial.

MECHANISM OF ACTION - Vaccine (claimed); gene therapy.

No biological data is given.

USE - (III) is useful as a vaccine composition, as a pharmaceutical, or in the manufacture of a medicament for the treatment or prevention of **infection** due to **Chlamydia**, preferably *C. pneumoniae* (claimed). (I) is useful for detecting *C. pneumoniae* in a sample. (II) is useful in

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polymerase chain reaction (PCR), branched DNA probe assay or blotting techniques for determining the presence of cDNA or mRNA.  
Dwg.0/191

L18 ANSWER 5 OF 26 HCPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 3  
ACCESSION NUMBER: 2001:936269 HCPLUS  
DOCUMENT NUMBER: 136:213285  
TITLE: Genomic approach for analysis of surface proteins in **Chlamydia pneumoniae**  
AUTHOR(S): Montigiani, Silvia; Falugi, Fabiana; Scarselli, Maria; Finco, Oretta; Petracca, Roberto; Galli, Giuliano; Mariani, Massimo; Manetti, Roberto; Agnusdei, Mauro; Cevenini, Roberto; Donati, Manuela; Nogarotto, Renzo; Norais, Nathalie; Garaguso, Ignazio; Nuti, Sandra; Saletti, Giulietta; Rosa, Domenico; **Ratti, Giulio**; Grandi, Guido  
CORPORATE SOURCE: Chiron SpA, Siena, 53100, Italy  
SOURCE: Infection and Immunity (2002), 70(1), 368-379  
CODEN: INFIBR; ISSN: 0019-9567  
PUBLISHER: American Society for Microbiology  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB **Chlamydia pneumoniae**, a human pathogen causing respiratory infections and probably contributing to the development of atherosclerosis and heart disease, is an obligate intracellular parasite which for replication needs to productively interact with and enter human cells. Because of the intrinsic difficulty in working with *C. pneumoniae* and in the absence of reliable tools for its genetic manipulation, the mol. definition of the **chlamydial** cell surface is still limited, thus leaving the mechanisms of **chlamydial** entry largely unknown. In an effort to define the surface protein organization of *C. pneumoniae*, we have adopted a combined genomic-proteomic approach based on (i) *in silico* prediction from the available genome sequences of peripherally located proteins, (ii) heterologous expression and purification of selected proteins, (iii) production of mouse immune sera against the recombinant proteins to be used in Western blotting and fluorescence-activated cell sorter (FACS) analyses for the identification of surface antigens, and (iv) mass spectrometry anal. of two-dimensional electrophoresis (2DE) maps of **chlamydial** protein exts. to confirm the presence of the FACS-pos. antigens in the **chlamydial** cell. Of the 53 FACS-pos. sera, 41 recognized a protein species with the expected size on Western blots, and 28 of the 53 antigens shown to be surface-exposed by FACS were identified on 2DE maps of elementary-body exts. This work represents the first systematic attempt to define surface protein organization in *C. pneumoniae*.

REFERENCE COUNT: 58 THERE ARE 58 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 6 OF 26 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 4  
ACCESSION NUMBER: 2001:387223 BIOSIS  
DOCUMENT NUMBER: PREV200100387223  
TITLE: **Chlamydia trachomatis** serotype D genes.

Searcher : Shears 308-4994

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AUTHOR(S): **Ratti, Giulio** [Inventor, Reprint author];  
Comanducci, Maurizio [Inventor]; Tecce, Mario F.  
[Inventor]; Giuliani, Marzia M. [Inventor]  
CORPORATE SOURCE: Siena, Italy  
ASSIGNEE: Scalvo SpA, Italy  
PATENT INFORMATION: US 6248563 June 19, 2001  
SOURCE: Official Gazette of the United States Patent and  
Trademark Office Patents, (June 19, 2001) Vol. 1247,  
No. 3. e-file.  
CODEN: OGUP7. ISSN: 0098-1133.

DOCUMENT TYPE: Patent  
LANGUAGE: English  
ENTRY DATE: Entered STN: 15 Aug 2001  
Last Updated on STN: 19 Feb 2002

AB A plasmid isolated from **Clamydia trachomatis** is described, which comprises 8 genes encoding proteins useful in the formulation of vaccines or diagnostic test for determining the bacterium or specific antibodies generated during **C. trachomatis infections**; in particular the recombinant fusion MS2-pgp3D protein is described comprising polypeptidic sequences encoded by pCT and immunogenic in the course of **infections** in man. A method for preparing said protein in *E. coli* further described.

L18 ANSWER 7 OF 26 USPATFULL on STN  
ACCESSION NUMBER: 2001:47842 USPATFULL  
TITLE: DNA molecules encoding pgp3 protein from  
**Chlamydia trachomatis**  
INVENTOR(S): **Ratti, Giulio**, Siena, Italy  
PATENT ASSIGNEE(S): Chiron SpA, Italy (non-U.S. corporation)

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6210968	B1	20010403
APPLICATION INFO.:	US 1995-465465		19950605 (8)
RELATED APPLN. INFO.:	Division of Ser. No. US 1994-229980, filed on 19 Apr 1994		
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	Granted		
PRIMARY EXAMINER:	Hobbs, Lisa J.		
LEGAL REPRESENTATIVE:	Blackburn, Robert P., Harbin, Alisa A. Woodcock Washburn Kurtz Mackiewicz & Norris LLP		
NUMBER OF CLAIMS:	4		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	1 Drawing Figure(s); 1 Drawing Page(s)		
LINE COUNT:	1596		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.  
AB A new recombinant form of the plasmid-encoded protein pgp3 from **C. trachomatis**, serotype D, was purified by ion exchange column chromatography and shown to be suitable for quantitative immunoassay on clinical samples in an ELISA format.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L18 ANSWER 8 OF 26 HCPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 5  
ACCESSION NUMBER: 2000:441819 HCPLUS  
DOCUMENT NUMBER: 133:72938  
TITLE: **Chlamydia trachomatis**  
antigens

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INVENTOR(S): **Ratti, Giulio**  
PATENT ASSIGNEE(S): Chiron S.p.A., Italy  
SOURCE: PCT Int. Appl., 25 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000037494	A2	20000629	WO 1999-IB2065	19991217
WO 2000037494	A3	20001012		
W: CA, JP, US				
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
CA 2355876	AA	20000629	CA 1999-2355876	19991217
EP 1140997	A2	20011010	EP 1999-958455	19991217
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2002534062	T2	20021015	JP 2000-589563	19991217
PRIORITY APPLN. INFO.:			GB 1998-28000 A	19981218
			WO 1999-IB2065 W	19991217

AB Proteins encoded by **Chlamydia trachomatis** which are immunogenic in humans as a consequence of **infection** have been identified using Western blots of two-dimensional electrophoretic maps. Several known immunogens were identified, as were proteins not previously known to be immunogens, and proteins not previously reported as expressed gene products.

L18 ANSWER 9 OF 26 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN DUPLICATE 6

ACCESSION NUMBER: 2001:197559 BIOSIS  
DOCUMENT NUMBER: PREV200100197559  
TITLE: Recombinant **Chlamydia trachomatis** pgp3 fusion protein.  
AUTHOR(S): Ratti, Giulio [Inventor, Reprint author];  
Comanducci, Maurizio [Inventor]; Tecce, Mario F.  
[Inventor]; Giuliani, Marzia M. [Inventor]  
CORPORATE SOURCE: Siena, Italy  
ASSIGNEE: Chiron S.p.A., Siena, Italy  
PATENT INFORMATION: US 6110705 August 29, 2000  
SOURCE: Official Gazette of the United States Patent and  
Trademark Office Patents, (Aug. 29, 2000) Vol. 1237,  
No. 5. e-file.  
CODEN: OGUPE7. ISSN: 0098-1133.

DOCUMENT TYPE: Patent  
LANGUAGE: English  
ENTRY DATE: Entered STN: 25 Apr 2001  
Last Updated on STN: 18 Feb 2002

AB A plasmid isolated from **Chlamydia trachomatis** is described, which comprises 8 genes encoding proteins useful in the formation of vaccines or diagnostic test for determining the bacterium or specific antibodies generated during C. **trachomatis infections**. In particular, the recombinant fusion protein MS2-pgp3D is described, which comprises polypeptide sequences encoded by pCT and is immunogenic in the course of **infections** in man. A method for preparing the

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recombinant fusion protein MS2-pgp3D in E. coli is also described.

L18 ANSWER 10 OF 26 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on  
STN DUPLICATE 7

ACCESSION NUMBER: 2001:193995 BIOSIS  
DOCUMENT NUMBER: PREV200100193995  
TITLE: **Chlamydia trachomatis** serotype D  
proteins.

AUTHOR(S): Ratti, Givlio [Inventor, Reprint author];  
Comanducci, Maurizio [Inventor]; Tecce, Mario F.  
[Inventor]; Giuliani, Marzia M. [Inventor]

CORPORATE SOURCE: Siena, Italy  
ASSIGNEE: Sclavo SpA, Italy

PATENT INFORMATION: US 6096519 August 01, 2000

SOURCE: Official Gazette of the United States Patent and  
Trademark Office Patents, (Aug. 1, 2000) Vol. 1237,  
No. 1. e-file.  
CODEN: OGUP7. ISSN: 0098-1133.

DOCUMENT TYPE: Patent

LANGUAGE: English

ENTRY DATE: Entered STN: 20 Apr 2001

Last Updated on STN: 18 Feb 2002

AB A plasmid isolated from **Clamydia trachomatis** is described,  
which comprises 8 genes encoding proteins useful in the formulation  
of vaccines or diagnostic test for determining the bacterium or  
specific antibodies generated during **C. trachomatis**  
**infections**; in particular the recombinant fusion MS2-pgp3D  
protein is described comprising polypeptidic sequences encoded by  
pCT and immunogenic in the course of **infections** in man. A  
method for preparing said protein in E. coli further described.

L18 ANSWER 11 OF 26 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 8

ACCESSION NUMBER: 1999:577186 HCAPLUS

DOCUMENT NUMBER: 131:211217

TITLE: Identification of immunoreactive proteins of  
**Chlamydia trachomatis** by

AUTHOR(S): Western blot analysis of a two-dimensional  
electrophoresis map with patient sera  
Sanchez-Campillo, Maria; Bini, Luca; Comanducci,  
Maurizio; Raggiaschi, Roberto; Marzocchi,  
Barbara; Pallini, Vitaliano; Ratti,  
Giulio

CORPORATE SOURCE: IRIS Reserach Center, Siena, I-53100, Italy

SOURCE: Electrophoresis (1999), 20(11), 2269-2279

CODEN: ELCTDN; ISSN: 0173-0835

PUBLISHER: Wiley-VCH Verlag GmbH

DOCUMENT TYPE: Journal

LANGUAGE: English

AB Western blots of two-dimensional electrophoretic maps of proteins  
from **Chlamydia trachomatis** were probed with sera  
from 17 seropos. patients with genital inflammatory disease.  
Immunoblot patterns (comprising 28 to 2 spots, average 14.8) were  
different for each patient; however, antibodies against a  
spot-cluster due to the **chlamydia**-specific antigen outer  
membrane protein-2 (OMP2) were observed in all sera. The next most  
frequent group of antibodies (15/17; 88%) recognized the hsp60  
GroEL-like protein, described as immunopathogenic in  
**chlamydial infections**. Reactivity to the major

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surface-exposed and variable antigen major outer membrane protein (MOMP) was observed at a relatively lower frequency (13/17; 76%). The hsp70 DnaK-like protein was also frequently recognized (11/17; 64.7%) in this patient group. Besides the above confirmatory findings, the study detected several new immunoreactive proteins, with frequencies ranging from 11/17 to 1/17. Some were characterized also by N-terminal amino acid sequencing and homol. searches. Amongst these were a novel outer membrane protein (OmpB) and, interestingly, five conserved bacterial proteins: four (23%) sera reacted with the RNA polymerase alpha-subunit, five (29%) recognized the ribosomal protein S1, eight (47%) the protein elongation factor EF-Tu, seven (41%) a putative stress-induced protease of the HtrA family, and seven sera (41%) the ribosomal protein L7/L12. Homologs of the last two proteins were shown to confer protective immunity in other bacterial infections. The data show that immunol. sensitization processes commonly thought to play a role in **chlamydial** pathogenicity may be sustained not only by the hsp60 GroEI-like protein, but also by other conserved bacterial antigens, some of which may be also considered as potential vaccine candidates.

REFERENCE COUNT: 32 THERE ARE 32 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L18 ANSWER 12 OF 26 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 1999:535766 BIOSIS

DOCUMENT NUMBER: PREV199900535766

TITLE: Humoral immune response to **Chlamydia trachomatis** in reactive arthritis analysed by two dimensional immunoblotting, and compared with the T-cell response.

AUTHOR(S): Raggiaschi, Roberto [Reprint author]; Portig, Irene; Ratti, Giulio; Pallini, Vitaliano; Gaston, Hill J. S.

CORPORATE SOURCE: Cambridge, UK

SOURCE: Arthritis and Rheumatism, (Sept., 1999) Vol. 42, No. 9 SUPPL., pp. S339. print.

Meeting Info.: 63rd Annual Scientific Meeting of the American College of Rheumatology and the 34th Annual Scientific Meeting of the Association of Rheumatology Health Professionals. Boston, Massachusetts, USA. November 13-17, 1999.

CODEN: ARHEAW. ISSN: 0004-3591.

DOCUMENT TYPE: Conference; (Meeting)  
Conference; Abstract; (Meeting Abstract)  
Conference; (Meeting Poster)

LANGUAGE: English

ENTRY DATE: Entered STN: 10 Dec 1999

Last Updated on STN: 10 Dec 1999

L18 ANSWER 13 OF 26 USPATFULL on STN

ACCESSION NUMBER: 97:40647 USPATFULL

TITLE: Detection of antibodies against **Chlamydia trachomatis** pgp3 antigen in patient sera by enzyme-linked immunosorbent assay

INVENTOR(S): Ratti, Giulio, Siena, Italy

PATENT ASSIGNEE(S): Biocine S.p.A., Italy (non-U.S. corporation)

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	NUMBER	KIND	DATE
PATENT INFORMATION:	US 5629167		19970513
APPLICATION INFO.:	US 1994-229980		19940419 (8)
DOCUMENT TYPE:	Utility		
FILE SEGMENT:	Granted		
PRIMARY EXAMINER:	Knode, Marian C.		
ASSISTANT EXAMINER:	Duffy, Patricia A.		
LEGAL REPRESENTATIVE:	Woodcock, Washburn, Kurtz, Mackiewicz & Norris, McClung, Barbara G., Blackburn, Robert P.		
NUMBER OF CLAIMS:	2		
EXEMPLARY CLAIM:	1		
NUMBER OF DRAWINGS:	1 Drawing Figure(s); 1 Drawing Page(s)		
LINE COUNT:	1258		

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB A new recombinant form of the plasmid-encoded protein pgp3 from *C. trachomatis*, serotype D, was purified by ion exchange column chromatography and shown to be suitable for quantitative immunoassay on clinical samples in an ELISA format.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L18 ANSWER 14 OF 26 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 1997:339355 BIOSIS  
DOCUMENT NUMBER: PREV199799638558  
TITLE: Characterization of a new isolate of *Chlamydia trachomatis* which lacks the common plasmid and has properties of biovar trachoma.

AUTHOR(S): Farencena, Aldo; Comanducci, Maurizio; Donati, Manuela; Ratti, Giulio [Reprint author]; Cevenini, Roberto

CORPORATE SOURCE: IRIS Res. Cent., Chiron Vaccines, 1 Via Fiorentina, 53110 Seina, Italy

SOURCE: Infection and Immunity, (1997) Vol. 65, No. 7, pp. 2965-2969.

CODEN: INFIBR. ISSN: 0019-9567.

DOCUMENT TYPE: Article

LANGUAGE: English

ENTRY DATE: Entered STN: 11 Aug 1997  
Last Updated on STN: 11 Aug 1997

AB A *Chlamydia trachomatis* urethral isolate, alpha/95, yielding pgp3-negative but otherwise normal inclusions by immunofluorescence also gave negative results when pCT-homologous DNA was searched by PCR and Southern blotting. omp-1 sequence analysis identified alpha/95 as a new genotype B variant. These findings confirm that pCT is not required for chlamydial growth in vitro.

L18 ANSWER 15 OF 26 MEDLINE on STN

ACCESSION NUMBER: 96016281 MEDLINE

DOCUMENT NUMBER: 96016281 PubMed ID: 7564710

TITLE: New chlamydial antigen as a serological marker in HIV infection.

AUTHOR: Ratti G; Comanducci M; Orfila J; Sueur J M;  
Gommeaux A

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SOURCE: LANCET, (1995 Sep 30) 346 (8979) 912.  
Journal code: 2985213R. ISSN: 0140-6736.

PUB. COUNTRY: ENGLAND: United Kingdom

DOCUMENT TYPE: Letter

LANGUAGE: English

FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals;  
AIDS

ENTRY MONTH: 199510

ENTRY DATE: Entered STN: 19951227  
Last Updated on STN: 19970203  
Entered Medline: 19951027

L18 ANSWER 16 OF 26 WPIDS COPYRIGHT 2003 THOMSON DERWENT on STN

ACCESSION NUMBER: 1995-373801 [48] WPIDS

DOC. NO. CPI: C1995-161993

TITLE: Recombinant *C. trachomatis* pgp3 protein -  
used for vaccinating against or treating *C.*  
*trachomatis* infection or for  
immuno-diagnosis.

DERWENT CLASS: B04 D16

INVENTOR(S): RATTI, G

PATENT ASSIGNEE(S): (BIOC-N) BIOCINE SPA; (CHIR) CHIRON SPA; (CHIR-N)  
CHIRON SPA

COUNTRY COUNT: 65

PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 9528487	A2	19951026 (199548)*	EN	71	
RW: AT BE CH DE DK ES FR GB GR IE IT KE LU MC MW NL OA PT SD SE SZ UG					
W: AM AT AU BB BG BR BY CA CH CN CZ DE DK EE ES FI GB GE HU IS JP KE KG KP KR KZ LK LR LT LU LV MD MG MN MW MX NO NZ PL PT RO RU SD SE SG SI SK TJ TM TT UA UG US UZ VN					
AU 9522227	A	19951110 (199607)			
EP 756630	A1	19970205 (199711)	EN		
R: AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE					
US 5629167	A	19970513 (199725)		13	
JP 10503922	W	19980414 (199825)		65	
US 6210968	B1	20010403 (200120)			
EP 756630	B1	20030723 (200356)	EN		
DE 69531345	E	20030828 (200364)			

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 9528487	A2	WO 1995-IB310	19950418
AU 9522227	A	AU 1995-22227	19950418
EP 756630	A1	EP 1995-915295	19950418
		WO 1995-IB310	19950418
US 5629167	A	US 1994-229980	19940419
JP 10503922	W	JP 1995-526847	19950418
		WO 1995-IB310	19950418
US 6210968	B1 Div ex	US 1994-229980	19940419
		US 1995-465465	19950605
EP 756630	B1	EP 1995-915295	19950418

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DE 69531345 E	WO 1995-IB310	19950418
	DE 1995-631345	19950418
	EP 1995-915295	19950418
	WO 1995-IB310	19950418

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 9522227	A Based on	WO 9528487
EP 756630	A1 Based on	WO 9528487
JP 10503922	W Based on	WO 9528487
EP 756630	B1 Based on	WO 9528487
DE 69531345	E Based on	EP 756630
	Based on	WO 9528487

PRIORITY APPLN. INFO: US 1994-229980 19940419; US 1995-465465  
19950605

AN 1995-373801 [48] WPIDS  
AB WO 9528487 A UPAB: 19990316

A recombinant **Chlamydia trachomatis** (CT) pgp3 protein (I) and derivs. and fragments are claimed. Also claimed are: (1) a vector comprising a recombinant polynucleotide encoding (I); (2) a host cell, pref. E.coli, transformed with a vector as in (1); and (3) a method for producing (I) by culturing the cells of (2) and isolating (I).

USE - The recombinant CT pgp3 protein can be used for vaccinating against CT infection or treating such infection in a human or animal (claimed). It can also be used in immunodiagnostic assays, pref. an ELISA (claimed).

ADVANTAGE - The recombinant pgp3 protein can be produced in the periplasm of E.coli, greatly facilitating purification. It can be produced in a conformation capable of recognition by antibodies in human serum.

Dwg.0/4

ABEQ US 5629167 A UPAB: 19970619

An enzyme-linked immunosorbent assay for the detection of anti-**Chlamydia trachomatis** pgp3 antibody in a patient sample comprising the following steps:

- (a) contacting the patient sample with a recombinant **Chlamydia trachomatis** pgp3 protein consisting of the amino acid sequence of native pgp3 protein bound to a solid support for a time sufficient to effect the binding of antibody to said bound protein;
- (b) detecting the binding of said antibody to said bound protein;
- (c) comparing the amount of antibody bound in step (b) to a control, wherein an increase over the control indicates the presence of anti-**Chlamydia trachomatis** pgp3 antibody.

Dwg.0/1

L18 ANSWER 17 OF 26 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 95297080 EMBASE

DOCUMENT NUMBER: 1995297080

TITLE: New chlamydial antigen as a serological marker in HIV infection [28].

AUTHOR: Ratti G.; Comanducci M.; Orfila J.; Sueur

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J.-M.; Gommeaux A.  
CORPORATE SOURCE: Laboratories HCM Chlamydia Network, European  
Community, Blobanque de Picardie, Amiens, France  
SOURCE: Lancet, (1995) 346/8979 (912).  
ISSN: 0140-6736 CODEN: LANCAO  
COUNTRY: United Kingdom  
DOCUMENT TYPE: Journal; Letter  
FILE SEGMENT: 004 Microbiology  
006 Internal Medicine  
026 Immunology, Serology and Transplantation  
029 Clinical Biochemistry  
LANGUAGE: English

L18 ANSWER 18 OF 26 SCISEARCH COPYRIGHT 2003 THOMSON ISI on STN  
ACCESSION NUMBER: 95:671603 SCISEARCH  
THE GENUINE ARTICLE: RX196  
TITLE: NEW CHLAMYDIAL ANTIGEN AS A SEROLOGICAL  
MARKER IN HIV-INFECTION  
AUTHOR: RATTI G (Reprint); COMANDUCCI M; ORFILA J;  
SUEUR J M; GOMMEAUX A  
CORPORATE SOURCE: EUROPEAN COMMUNITY BIOBANQUE PICARDIE, HCM CHLAMYDIA  
NETWORK LABS, AMIENS, FRANCE (Reprint); IRIS RES  
CTR, CHIRON BIOCINE SIENA, ITALY  
COUNTRY OF AUTHOR: FRANCE; ITALY  
SOURCE: LANCET, (30 SEP 1995) Vol. 346, No. 8979, pp. 912.  
ISSN: 0099-5355.  
DOCUMENT TYPE: Letter; Journal  
FILE SEGMENT: LIFE; CLIN  
LANGUAGE: ENGLISH  
REFERENCE COUNT: 3

L18 ANSWER 19 OF 26 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 9  
ACCESSION NUMBER: 1995:230263 HCAPLUS  
DOCUMENT NUMBER: 122:7457  
TITLE: Humoral immune response to plasmid protein pgp3  
in patients with **Chlamydia trachomatis** infection  
AUTHOR(S): Comanducci, M.; Manetti, R.; Bini, L.; Santucci,  
A.; Pallini, V.; Cevenini, R.; Sueur, J. M.;  
Orfila, J.; Ratti, G.  
CORPORATE SOURCE: Immunobiological Res. Inst., Siena, Italy  
SOURCE: Infection and Immunity (1994), 62(12), 5491-7  
CODEN: INFIBR; ISSN: 0019-9567  
PUBLISHER: American Society for Microbiology  
DOCUMENT TYPE: Journal  
LANGUAGE: English  
AB We identified, by two-dimensional electrophoretic anal. and  
microsequencing, a protein of **Chlamydia trachomatis** elementary bodies which corresponds to the  
polypeptide (pgp3) encoded by open reading frame 3 (ORF3). Amino  
acid anal. showed that the first residue (Gly) found in the native  
protein is the one encoded by the second ORF3 codon, implying a  
typical bacterial removal of the first Met residue. Relatively  
large amts. of recombinant pgp3 (r-pgp3) in a stable, water-soluble  
form were obtained by overexpressing ORF3 in Escherichia coli and  
purifying the product from periplasmic exts. under nondenaturing  
conditions. These r-pgp3 prepns. allowed specific detection of  
anti-pgp3 antibodies by ELISA. Anal. of a group of 170 sera from

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healthy blood donors and from patients who were seropos. or -neg. for *C. trachomatis* and *Chlamydia pneumoniae* showed that an immune response to pgp3 occurs in the majority (ca. 81%) of patients with sexually transmitted diseases who are seropos. for *C. trachomatis* and generally correlates with the response to cell surface antigens. No reaction between r-pgp3 and *C. pneumoniae*-pos. sera was detected.

L18 ANSWER 20 OF 26 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 10  
ACCESSION NUMBER: 1993:618588 HCAPLUS  
DOCUMENT NUMBER: 119:218588  
TITLE: Expression of a plasmid gene of  
*Chlamydia trachomatis* encoding  
a novel 28 kDa antigen  
AUTHOR(S): Comanducci, Maurizio; Cevenini, Roberto; Moroni,  
Alessandra; Giuliani, Marzia M.; Ricci, Stefano;  
Scarlato, Vincenzo; **Ratti, Giulio**  
CORPORATE SOURCE: Immunobiol. Res. Inst. Siena, Siena, 53100,  
Italy  
SOURCE: Journal of General Microbiology (1993), 139(5),  
1083-92  
CODEN: JGMIAN; ISSN: 0022-1287  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB With the purpose of investigating the possibility of a role of plasmid pCT in *C. trachomatis* pathogenicity, the authors examined the expression of an ORF (ORF3), potentially encoding a 28 kDa polypeptide (pgp3). Anal. of RNA extracted from *chlamydia*-infected Vero cells detected ORF3-specific transcripts, from 20 h post-infection onwards, mainly as discrete RNA species of 1390 nucleotides comprising the downstream ORF4 sequence. ORF3 DNA was cloned and expressed in *Escherichia coli* as a 39 kDa fusion protein (MS2/pgp3). Antibodies raised against purified MS2/pgp3 specifically recognized a 38 kDa protein on Western blots of protein from purified *chlamydial* elementary bodies (EBs). The same antibodies detected *chlamydial* inclusions in methanol-fixed infected cells by immunofluorescence. Western blot anal. of EBs extracted with 2% Sarkosyl showed that a large proportion of the 28 kDa antigen is associated with the detergent-insol. (membrane) fraction. Antibodies recognizing pgp3 epitopes were detected in sera from patients with *chlamydial* infections, but not in sero-neg. control sera. Apparently, pCT may provide a function related to *chlamydial* cell physiol.

L18 ANSWER 21 OF 26 HCAPLUS COPYRIGHT 2003 ACS on STN DUPLICATE 11  
ACCESSION NUMBER: 1993:95597 HCAPLUS  
DOCUMENT NUMBER: 118:95597  
TITLE: Plasmid pCTD of *Chlamydia trachomatis* serotype D, its isolation and sequencing  
INVENTOR(S): **Ratti, Giulio**; Comanducci, Maurizio;  
Tecce, Mario F.; Giuliani, Marzia M.  
PATENT ASSIGNEE(S): Sclavo S.p.A., Italy  
SOURCE: Eur. Pat. Appl., 40 pp.  
CODEN: EPXXDW  
DOCUMENT TYPE: Patent  
LANGUAGE: English

09/868293

FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 499681	A1	19920826	EP 1991-106110	19910417
EP 499681	B1	19990609		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE				
AT 181104	E	19990615	AT 1991-106110	19910417
ES 2136062	T3	19991116	ES 1991-106110	19910417
US 6110705	A	20000829	US 1995-444189	19950518
US 6248563	B1	20010619	US 1995-468544	19950606
US 6096519	A	20000801	US 1997-969644	19971113
PRIORITY APPLN. INFO.:			IT 1991-MI314	A 19910207
			US 1991-661820	B1 19910228
			US 1992-991512	B3 19921217
			US 1994-180528	B1 19940112
			US 1995-444189	A3 19950518
			US 1995-467152	B3 19950606

AB The plasmid pCTD (7.5 kb) of *C. trachomatis* serotype D is cloned and sequenced, and the open reading frame ORF3 is expressed in Escherichia coli. A BamHI DNA library of the elemental body of *C. trachomatis* serotype D obtained from a patient with non-gonococcal urethritis was constructed in pUC18. The library was screened with 3 synthetic DNA probes (sequences given) to find pCTD as a 7.5-kb insert. ORF3 of the pCTD was amplified by PCR, cloned into pEX34A, and expressed in E. coli as a fusion protein with RNA polymerase fragment of bacteriophage MS2. Polyclonal and monoclonal antibodies were raised against the fusion protein by standard methods. Also shown was the use of the fusion protein MS2-pgp3 for detection of *Chlamydia infection*.

L18 ANSWER 22 OF 26 MEDLINE on STN DUPLICATE 12  
ACCESSION NUMBER: 91310797 MEDLINE  
DOCUMENT NUMBER: 91310797 PubMed ID: 1856288  
TITLE: Detection of *Chlamydia trachomatis*  
DNA in patients with non-gonococcal urethritis using  
the polymerase chain reaction.  
COMMENT: Erratum in: J Clin Pathol 1992 Jan;45(1):92  
AUTHOR: Ratti G; Moroni A; Cevenini R  
CORPORATE SOURCE: Sclavo Research Centre, Siena, Italy.  
SOURCE: JOURNAL OF CLINICAL PATHOLOGY, (1991 Jul) 44 (7)  
564-8.  
Journal code: 0376601. ISSN: 0021-9746.  
PUB. COUNTRY: ENGLAND: United Kingdom  
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
LANGUAGE: English  
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals  
ENTRY MONTH: 199108  
ENTRY DATE: Entered STN: 19910913  
Last Updated on STN: 19910913  
Entered Medline: 19910827

AB A practical protocol using the polymerase chain reaction (PCR) was designed for detecting *Chlamydia trachomatis* in clinical samples. DNA was extracted from material collected on urethral swabs and used as substrate for the PCR. The target was a 600 basepair DNA segment of the multicopy plasmid that is common to all strains of the bacterium. Negative samples were checked for

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loss of DNA or presence of polymerase inhibitors by a second PCR, targeted to a conserved segment of the human genome. The whole procedure was tested on 216 men with non-gonococcal urethritis (NGU). All patients were independently assessed by tissue culture isolation (60 positive samples) and a commercial immunoenzymatic assay. The PCR protocol, while sufficiently simple for routine application, was reliable and, for the diagnosis of urethritis, at least as good as tissue culture isolation.

L18 ANSWER 23 OF 26 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 1991:410963 BIOSIS  
DOCUMENT NUMBER: PREV199192077928; BA92:77928  
TITLE: DEVELOPMENT OF TRANSPLANTABLE ASCITES TUMORS WHICH CONTINUOUSLY PRODUCE POLYCLONAL ANTIBODIES IN PRISTANE PRIMED BALB-C MICE IMMUNIZED WITH BACTERIAL ANTIGENS AND COMPLETE FREUND'S ADJUVANT.  
AUTHOR(S): CEVENINI R [Reprint author]; SAMBRI V; PILERI S; RATTI G; LA PLACA M  
CORPORATE SOURCE: INST MICROBIOLOGY, OSPEDALE S ORSOLA, 9 VIA MASSARENTI, 40138 BOLOGNA, ITALY  
SOURCE: Journal of Immunological Methods, (1991) Vol. 140, No. 1, pp. 111-118.  
CODEN: JIMMBG. ISSN: 0022-1759.  
DOCUMENT TYPE: Article  
FILE SEGMENT: BA  
LANGUAGE: ENGLISH  
ENTRY DATE: Entered STN: 11 Sep 1991  
Last Updated on STN: 11 Sep 1991

AB Bacterial immunogens (whole cells of *Borrelia burgdorferi*, elementary bodies of *Chlamydia trachomatis* and purified proteins of 22 and 24 kDa of *Borrelia hermsii*) were emulsified with an excess of complete Freund's adjuvant and injected (i.p.) on days 0, 7, 14, and 21, into BALB/c mice treated with pristane on day 6. This procedure induced the development of antibody-producing ascites tumours which could be serially transplanted in pristane-conditioned mice. Ascites tumours continued to yield a consistent amount of specific polyclonal antibody after ten serial transplants. The method described appears to be particularly useful for the production of a large amount of antibody when only small amounts of immunogen are available.

L18 ANSWER 24 OF 26 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER: 1990:413915 BIOSIS  
DOCUMENT NUMBER: PREV199090074716; BA90:74716  
TITLE: DIVERSITY OF THE CHLAMYDIA-TRACHOMATIS COMMON PLASMID IN BIOVARS WITH DIFFERENT PATHOGENICITY.  
AUTHOR(S): COMANDUCCI M [Reprint author]; RICCI S; CEVENINI R; RATTI G  
CORPORATE SOURCE: SCLAVO RES CENTRE, VIA FIORENTINA 1, 53100, SIENA ITALY  
SOURCE: Plasmid, (1990) Vol. 23, No. 2, pp. 149-154.  
CODEN: PLSMDX. ISSN: 0147-619X.  
DOCUMENT TYPE: Article  
FILE SEGMENT: BA  
LANGUAGE: ENGLISH

09/868293

ENTRY DATE:            Entered STN: 17 Sep 1990  
                      Last Updated on STN: 17 Sep 1990

AB    The 7.5-kb plasmid of **Chlamydia trachomatis** (CT) is believed to encode essential genes and might have a role in CT pathogenicity. Accordingly, analysis of plasmid-linked mutation in isolates from biovars with different pathogenic properties should help in identifying which plasmid-encoded genes, if any, may be involved in modulating virulence. For this purpose, this plasmid present in a low-virulence isolate (trachoma biovar, serotype D) was cloned and sequenced. Nucleotide changes were experimentally checked against the sequence of the plasmid variant from the highly virulent strain L2/434/Bu (LGV biovar). By aligning our data with two published sequences of different trachoma and LGV variants a general consensus structure was determined comprising eight major open reading frames (ORF) and a number of points where there is consensus only between isolates of the same biovar (biovar-specific mutations). The degree of variation between different isolates is less than 1%. In particular, composition of serotype-D and -L2 plasmids shows mutations which are generally silent or lead to few (one to four), often conservative, amino acid changes in ORFs 1, 2, 4, 5, 6, and 7. The protein encoded by ORF8 is completely conserved. In contrast, the polypeptide variants encoded by ORF3 show nine amino acids changes, seven of which are due to biovar-specific mutations.

L18 ANSWER 25 OF 26 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN

ACCESSION NUMBER:      1989:72574 BIOSIS  
DOCUMENT NUMBER:        PREV198987036972; BA87:36972  
TITLE:                  THE STRUCTURE OF A PLASMID OF **CHLAMYDIA TRACHOMATIS** BELIEVED TO BE REQUIRED FOR GROWTH WITHIN MAMMALIAN CELLS.  
AUTHOR(S):              COMANDUCCI M [Reprint author]; RICCI S; RATTI G  
CORPORATE SOURCE:       SCLAVO RES CENTER, 53100 SIENA, ITALY  
SOURCE:                 Molecular Microbiology, (1988) Vol. 2, No. 4, pp. 531-538.  
CODEN:                 MOMIEE. ISSN: 0950-382X.

DOCUMENT TYPE:          Article  
FILE SEGMENT:           BA  
LANGUAGE:                ENGLISH  
OTHER SOURCE:           GENBANK-X07547  
ENTRY DATE:             Entered STN: 23 Jan 1989  
                      Last Updated on STN: 23 Jan 1989

AB    Sequence analysis of a 7.5 kb DNA plasmid isolated from **Chlamydia trachomatis** shows 8 open reading frames (ORFs) regularly spaced along most of the sequence. One of these ORFs encodes a 451-amino acid polypeptide highly homologous to the DnaB protein of *Escherichia coli*. A region between ORFs 6 and 7 contains a cluster of alternating ATs and a 22 bp sequence tandemly repeated 4 times, suggesting a replication control region. Several ORFs correspond to plasmid-specific polypeptides that have been described. Codons ending with A or T are more frequent, as might be expected from the high A/T content (64%) of the plasmid, and codon usage is similar to that of the *C. trachomatis* chromosomal gene, *omp1L2*.

L18 ANSWER 26 OF 26 EMBASE COPYRIGHT 2003 ELSEVIER INC. ALL RIGHTS

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RESERVED. on STN  
ACCESSION NUMBER: 88208812 EMBASE  
DOCUMENT NUMBER: 1988208812  
TITLE: Detection of **Chlamydia trachomatis**.  
in cytological samples by a biotinylated DNA probe  
test.  
AUTHOR: Garuti G.; Boselli F.; Genazzani A.; Comanducci M.;  
Silvestri S.; Ratti G.  
CORPORATE SOURCE: Department of Obstetrics and Gynecology, Modena  
University, 41100 Modena, Italy  
SOURCE: Cervix and the Lower Female Genital Tract, (1988) 6/2  
(135-140).  
ISSN: 0393-3512 CODEN: CLFTEH  
COUNTRY: Italy  
DOCUMENT TYPE: Journal  
FILE SEGMENT: 004 Microbiology  
010 Obstetrics and Gynecology  
LANGUAGE: English  
SUMMARY LANGUAGE: English  
AB An in situ DNA hybridization test which uses a **Chlamydia trachomatis** (CT) specific plasmid was employed to detect CT infection on cytological samples obtained from 160 patients. Eighteen (11.3%) samples were positive, and the subjects could be considered infected by CT. The tested procedure appears to be simple and rapid, and suitable for routine clinical application.

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## Cloning and Characterization of RNA Polymerase Core Subunits of *Chlamydia trachomatis* by Using the Polymerase Chain Reaction

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Received 4 January 1990/Accepted 19 June 1990

Taking advantage of sequence conservation of portions of the  $\alpha$ ,  $\beta$ , and  $\beta'$  subunits of RNA polymerase of bacteria and plant chloroplasts, we have designed degenerate oligonucleotides corresponding to these domains and used these synthetic DNA sequences as primers in a polymerase chain reaction to amplify DNA sequences from the chlamydial genome. The polymerase chain reaction products were used as a probe to recover the genomic fragments encoding the  $\beta$  subunit and the 5' portion of the  $\beta'$  subunit from a library of cloned murine *Chlamydia trachomatis* DNA. Similar attempts to recover the  $\alpha$  subunit were unsuccessful. Sequence analysis demonstrated that the  $\beta$  subunit of RNA polymerase was located between genes encoding the L7/L12 ribosomal protein and the  $\beta'$  subunit of RNA polymerase; this organization is reminiscent of the *rpoBC* operon of *Escherichia coli*. The *C. trachomatis*  $\beta$  subunit overproduced in *E. coli* was used as an antigen in rabbits to make a polyclonal antibody to this subunit. Although this polyclonal antibody specifically immunoprecipitated the  $\beta$  subunit from *Chlamydia*-infected cells, it did not immunoprecipitate core or holoenzyme. Immunoblots with this antibody demonstrated that the  $\beta$  subunit appeared early in infection.

*Chlamydia trachomatis* is an obligate intracellular parasite of eucaryotic cells (for reviews, see references 3, 26, and 27). This medically important gram-negative bacterium causes an array of ocular and genital disorders which rank among the most prevalent diseases of humans. Chlamydiae display a complex life cycle involving the sequential alternation of two different morphologic forms, the elementary body (EB) and the reticulate body (RB). The life cycle commences when the EB, the spore like metabolically inactive extracellular form, is taken up by the host eucaryotic cell. Upon binding to the host cell membrane and subsequent internalization into a host-derived endosome, the EB undergoes a striking morphologic transformation into the intracellular vegetative RB. The RB replicates by binary fission 100- to 1,000-fold while enclosed within this vacuole in the host cell cytoplasm. The newly replicated RBs subsequently redifferentiate into EBs that are released from the host cell, completing the intracellular life cycle.

Chlamydial development proceeds according to a strict program which clearly reflects the temporally regulated activation of specific sets of genes and at least superficially resembles the life cycle of the sporulating bacterium *Bacillus subtilis* (14, 15). The molecular basis of this developmentally regulated gene expression in chlamydiae is largely undefined, owing chiefly to the lack of convenient systems for gene transfer into this organism and to the paucity of information about the nature of the signals and machinery that govern chlamydial gene expression.

A major focus of our research has been to elucidate the *cis* elements and *trans*-acting factors that underlie the regulation of gene expression during this life cycle. In earlier studies, we and others have shown that chlamydial promoter sequences appear to be different from those of other prokaryotes (9, 24, 29; J. Engel and D. Ganem, in *Immune Recognition and Evasion: Molecular Aspects of Host-Parasite Interaction*, in press), and in fact, no chlamydial promoter

tested so far functions properly in *Escherichia coli* (24; Engel and Ganem, in press).

A fuller understanding of chlamydial gene regulation will require a more detailed characterization of chlamydial RNA polymerase, the central component of the transcriptional apparatus. Eubacterial RNA polymerases are multisubunit enzymes composed of  $\alpha$ ,  $\beta$ ,  $\beta'$ , and  $\sigma$  subunits (reviewed in reference 11). The core enzyme,  $\alpha\beta\beta'$ , is a nonspecific DNA-binding protein. Holoenzyme, formed by the association of the  $\sigma$  subunit with core enzyme, has the property of sequence-specific DNA recognition, permitting the specific binding of RNA polymerase to promoter sequences. The major  $\sigma$  subunit of *E. coli*,  $\sigma^{70}$ , is responsible for RNA polymerase binding to the basic promoter motif (TATAAT at -10 and TTGACA at -35). Direct biochemical purification of RNA polymerase from many bacterial species for use in *in vitro* transcription systems has been relatively straightforward, owing in part to the ability to grow large quantities of these microorganisms. Such an approach is not practical for chlamydiae; the poor growth of this bacterium in culture makes it exceedingly difficult to generate the necessary starting material for such large-scale purifications. Instead, it is likely that techniques developed for the isolation of rare protein species, such as immunoaffinity to antibody columns, may be necessary for chlamydial RNA polymerase characterization. To this end, we have turned our efforts towards cloning and overexpressing the subunits of chlamydial RNA polymerase in *E. coli* to facilitate its further purification for subsequent use in *in vitro* in the analysis of promoter structure and of protein factors important in the control of chlamydial gene expression.

Using a strategy that makes use of the polymerase chain reaction (PCR) to directly amplify related sequences from the chlamydial genome, we cloned and characterized a chlamydial homolog of  $\sigma^{70}$  (8). After identifying regions of the  $\alpha$ ,  $\beta$ , and  $\beta'$  subunits of *E. coli* RNA polymerase conserved in other organisms, we have now extended this PCR-based approach to the cloning of the  $\beta$  and  $\beta'$  subunits of RNA polymerase from a murine strain of *C. trachomatis*.

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## MATERIALS AND METHODS

**Reagents.** Products were obtained from the following sources and were used according to the manufacturer's specifications: restriction enzymes, bacterial alkaline phosphatase, and T4 DNA ligase, New England BioLabs, Inc. (Beverly, Mass.); T4 polynucleotide kinase, Boehringer Mannheim Biochemicals (Indianapolis, Ind.); DNA polymerase I, Pharmacia Fine Chemicals (Piscataway, N.J.);  $^{32}\text{P}$ -containing radioisotopes, Amersham Corp. (Arlington Heights, Ill.); [ $^{35}\text{S}$ ]methionine, ICN (Irvine, Calif.); *Thermus aquaticus* DNA polymerase, Cetus Corp (Emeryville, Calif.); SeaPlaque and Seakem agarose, FMC Bioproducts (Rockland, Maine); ampicillin, kanamycin, rifampin, protein A-Sepharose CL-4B, and DNase I, Sigma Chemical Co. (St. Louis, Mo.); dimethyl-3,3'-dithiobis-propionimidate (DTBP), Pierce Chemical Co. (Rockford, Ill.); and protein molecular weight markers, Bethesda Research Laboratories (Bethesda, Md.).

**Nucleic acid preparation and analysis.** Chlamydial DNA from the mouse pneumonitis (MoPn) strain of *C. trachomatis* was prepared as described previously (9). Human DNA was isolated from HeLa cells grown in culture (16). *E. coli* DNA was prepared from strain TG1 (T. J. Gibson, Ph.D. thesis, Cambridge University, Cambridge, England) as described before (16). Standard recombinant DNA methods were used for nucleic acid preparation and analysis (16). Restriction fragments were subcloned into a pGEM7zf (Promega, Madison, Wis.) plasmid vector. Southern blotting was carried out as described previously (9). Radioactive DNA probes were labeled by nick translation or by 5'-end labeling with T4 polynucleotide kinase (16).

**Synthetic oligonucleotides.** The following single-stranded oligonucleotide primers were synthesized by the Biomedical Resource Center at the University of California, San Francisco:  $\alpha$ 5' primer, CCGAATTCCA(TC)GA(AG)TA(TC)TC(AGTC)AC;  $\alpha$ 3' primer, GGCTCGAG(AGT)AT(AG)ATC(AGCT)GC(AGCT)GC(AGCT)G;  $\beta$ 5' primer, CC GAATTCAA(TC)ATGCA(AG)CG(ATGC)CA;  $\beta$ 3' primer, GGCTCGAG(AG)TC(TC)TC(AG)AA(AG)TT(AG)TA;  $\beta'$  5' primer, CCGAATTCA(TAC)CA(AG)GC(A GCT)TT and CCGAATTCCGG(AGCT)AA(AG)CG(ACG T)GT(AGCT)GA;  $\beta'$  3' primer, GGCTCGAG(AG)TC(AT)AA(AG)TC(AGCT)GC(AG)TT and GGCTCGAG(A G)AAG(ACGT)GC(TC)TG(ATG)AT(AGCT)CC.

**PCR.** The PCR was performed with a Cetus/Perkin-Elmer DNA thermocycler. Reaction mixtures (100  $\mu\text{l}$ ) contained 100 pmol of the 5' and 3' primers, all four dNTPs at 1 mM each, 50 mM KCl, 10 mM Tris chloride (pH 8.0), 2 mM MgCl<sub>2</sub>, 0.01% gelatin, 1  $\mu\text{g}$  of DNA, and 2.5 U of *T. aquaticus* DNA polymerase. The reaction mixture was overlaid with a drop of paraffin oil and subjected to 35 cycles consisting of a 2-min denaturation period at 94°C, a 2-min annealing period at 37°C, and a 2-min extension period at 72°C. After analysis of the PCR product on a 1.5% low-melting-point agarose gel (SeaPlaque), the amplification product was purified from the gel, followed by isolation with glass beads (GeneClean; Bio101, La Jolla, Calif.). The gel-purified product was digested with EcoRI and XbaI and cloned into pGEM7zf previously digested with EcoRI and XbaI, followed by treatment with bacterial alkaline phosphatase.

**Preparation and screening of a chlamydial DNA library.** Chlamydial DNA was digested with EcoRI and cloned into a pUC8 (Pharmacia, Piscataway, N.J.) vector previously cleaved with EcoRI and dephosphorylated with bacterial

alkaline phosphate. A total of 900 colonies were stabbed onto L-broth plates containing ampicillin (50  $\mu\text{g}/\text{ml}$ ). After overnight growth at 37°C, the plates were overlaid with Hybond filters (Amersham Corp., Arlington Heights, Ill.). Filters bearing colonies were soaked in 0.5 M NaOH-1.5 M NaCl, followed by 0.5 M Tris chloride (pH 8.0)-1.5 M NaCl. Following UV light cross-linking, the filters were hybridized as described previously (9) to a 5'-end-labeled probe made from the PCR product. The EcoRI fragments from the clones that hybridized to this probe were then recloned into the EcoRI site of pGEM7zf for further analysis.

**DNA sequencing.** The dideoxy chain termination method of DNA sequencing (23) was carried out on double-stranded fragments cloned into pGEM7zf with the Sequenase kit (United States Biochemical Corp., Cleveland, Ohio). Sequencing reactions were primed with oligonucleotides homologous to the T7 and SP6 promoters (Promega Biotech, Madison, Wis.) flanking the cloned inserts in the pGEM7zf vector. Most of the sequencing was carried out on one strand of the duplex only.

**Overproduction of bacterially encoded proteins.** Overproduction of proteins encoded by cloned genes of interest was accomplished by introducing the corresponding plasmid into a strain of *E. coli* harboring pGP1-2 [HMS262(pGP1-2)] (32), a pGEM-compatible plasmid that encodes the phage T7 RNA polymerase under control of a thermostable lambda repressor. The following plasmids were constructed in pGEM7zf (see Fig. 4B): pBETA (L7/L12, full-length  $\beta$  gene, and the 5' half of  $\beta'$ ), p291 (the 5' EcoRI-Clal fragment from pBETA), and p280 (5' EcoRI-NsiI fragment from pBETA). Qualitative induction of the plasmid-encoded gene product was carried out as follows. Strains were grown at 30°C in L-broth (16) containing kanamycin (50  $\mu\text{g}/\text{ml}$ ) and ampicillin (100  $\mu\text{g}/\text{ml}$ ) to an  $A_{600}$  of 0.6. Samples (1 ml) were pelleted and suspended in M9 minimal medium (16) containing thiamine and all amino acids except methionine. The cultures were then transferred to 42°C for 20 min, at which time rifampin (100  $\mu\text{g}/\text{ml}$ ) was added. Following further incubation at 42°C for 10 min, [ $^{35}\text{S}$ ]methionine was added (50  $\mu\text{Ci}/\text{ml}$ ), and the cultures were grown at 37°C for 30 min. The bacteria were pelleted and lysed in 100  $\mu\text{l}$  of Laemmli buffer (12) containing 5% (vol/vol)  $\beta$ -mercaptoethanol. Polyacrylamide gel electrophoresis (PAGE) of the protein products was carried out on 12% sodium dodecyl sulfate (SDS)-polyacrylamide slab gels (12), as modified (2).

Quantitative overproduction of the plasmid-encoded gene product was carried out similarly with the following modifications. One liter of HMS262(pGP1-2, p291) bacteria was grown and thermoinduced for protein expression in L-broth as described above. The bacterial cells were pelleted and frozen. After thawing, the bacteria were lysed by treatment with 110 mg of lysozyme in TEN buffer (10 mM Tris [pH 7.5], 1 mM EDTA, 0.1 M NaCl) on ice for 15 min, followed by the addition of Nonidet P-40 to 0.2% for 10 min. Then, 2 mg of DNase I was added, and the sample was stirred on ice for 1 h, followed by shearing of the remaining undigested DNA with a Polytron homogenizer/sonicator (model Ls10-35; Kinematica, Lucern, Switzerland) for 30 s. The sample was then centrifuged at 10,000  $\times g$  for 10 min, and the pellet was suspended by boiling in 3 ml of Laemmli buffer (12) containing 5% (vol/vol)  $\beta$ -mercaptoethanol for 20 min. The material was electrophoresed on a preparative 12% SDS-polyacrylamide gel (12). A strip from the gel was stained briefly in 10% acetic acid containing 0.25% Coomassie blue R250, and the band corresponding to the overexpressed  $\beta$  protein fragment was excised. This excised gel fragment was

immersed in 5 volumes of Laemmli lectrophoresis buffer (12) and broken into fragments by treatment with the Polytron homogenizer/sonicator for 30 s.  $\beta$ -Mercaptoethanol was added to 0.1%, and the protein was eluted from the gel by agitation at room temperature for 12 to 18 h. The acrylamide was pelleted by centrifugation, and the protein in the supernatant was precipitated by the addition of 3 volumes of methanol, followed by incubation on ice for 2 h. The precipitate was collected by centrifugation and suspended in phosphate-buffered saline (PBS) containing 0.1% SDS. A 150- $\mu$ g amount of the gel-purified protein was injected into a rabbit, followed by boosting. The antibody production was carried out by Caltag Corp. (Berkeley, Calif.).

**In vivo labeling of chlamydial proteins.** Chlamydia-infected HeLa cells were incubated in Dulbecco modified medium lacking methionine and cysteine in the presence of cycloheximide (50  $\mu$ g/ml) for 30 min, followed by pulse labeling with [ $^{35}$ S]methionine (100  $\mu$ Ci/ml) for 30 min at various times during infection. After extraction with lysis buffer (10% glycerol, 50 mM Tris chloride [pH 7.5], 150 mM NaCl, 0.2% Triton X-100, 1  $\mu$ g of aprotinin [Sigma Chemical Co., St. Louis, Mo.] per ml, 1 mM phenylmethylsulfonyl fluoride [Sigma], and 1 mM leupeptin [Sigma], samples were electrophoresed on 10% SDS-polyacrylamide gels.

**Immunoblot analysis and immunoprecipitations.** Immunoblots were carried out as described before (33), with 2% gelatin as a blocking agent. Preimmune or immune serum was used at a dilution of 1:200. Alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G (Promega) was used at a dilution of 1:7,500 for the second antibody reaction. To compete out antibodies to *E. coli* present in the rabbit antiserum, an *E. coli* protein lysate (Promega) was incubated at a concentration of 200  $\mu$ g/ml with a 1:800 dilution of the antiserum prior to incubation with the Western immunoblot of pBETA and its truncated derivatives expressed in *E. coli*.

Immunoprecipitations were performed as described previously (10) with the following modifications. Dishes (100 mm) or T75 flasks of subconfluent HeLa cells (uninfected or infected with chlamydiae for 12 h) were labeled with [ $^{35}$ S]methionine (500  $\mu$ Ci/ml) for an additional 8 h in methionine- and cysteine-free Dulbecco modified medium containing cycloheximide (50  $\mu$ g/ml). Cells were removed from the dishes by gentle agitation with 2 ml of lysis buffer, followed by a brief centrifugation of the supernatant at 12,000  $\times$  g. The supernatant was added to 20 to 50  $\mu$ l of protein A-Sepharose CL-4B beads along with preimmune or immune antiserum (5  $\mu$ l of serum per 10  $\mu$ l of beads) and rocked for 12 to 18 h at 4°C. The protein A-Sepharose beads were washed with RIPA buffer (50 mM Tris chloride [pH 7.5], 0.5 M NaCl, 20 mM EDTA, 0.2% Triton X-100, 0.05% SDS, 1% deoxycholate), followed by washes with PBS. The bound antigen was eluted in Laemmli sample buffer (12) containing 5% (vol/vol)  $\beta$ -mercaptoethanol and electrophoresed on 10% SDS-polyacrylamide slab gels. The radiolabeled product was visualized by fluorography.

For the *in vivo* cross-linking experiments, chlamydial infections were carried out as described above. The medium was removed, and 2 ml of DTBP (5 mg/ml in PBS) was added to the plates. Following gentle agitation at room temperature for 30 min, the DTBP-PBS was removed and the plates were washed three times with PBS. Immunoprecipitations were then carried out as above. For the *in vitro* cross-linking experiments, the cell lysates were adsorbed overnight to protein A-Sepharose-antibody. Then, 1/10 volume of DTBP (5 mg/ml in PBS) was added to the lysates, and the samples

were incubated by rocking at room temperature for 30 min. The immunoprecipitates were then washed as described above. Chlamydia-infected cells were exposed to a heat shock stress by incubating the dishes in a 45°C water bath for 10 min prior to cross-linking or extraction in lysis buffer. We have shown that these conditions induce a heat shock response in *C. trachomatis* (J. Engel, J. Pollack, E. Perara, and D. Ganem, unpublished data).

## RESULTS

**Cloning of the  $\beta$  subunit of chlamydial RNA polymerase.** The Dayhoff protein data base was searched for proteins with homology to the subunits of *E. coli* core RNA polymerase (6, 19–21). Significant homologies to the *E. coli* RNA polymerase  $\alpha$  chain were found in the RNA polymerases of vaccinia virus (7), *B. subtilis* (5), liverwort (18), and common tobacco plant chloroplast (28); significant homologies to the *E. coli* RNA polymerase  $\beta$  chain were found in the chloroplast RNA polymerase of the liverwort (18) and the common tobacco plant (17); and significant homologies to the *E. coli* RNA polymerase  $\beta'$  chain were found in the chloroplast RNA polymerase of liverwort (18), RNA polymerase II of *Drosophila melanogaster* (4) and *Saccharomyces cerevisiae* (1), and the vaccinia virus RNA polymerase (7) (Fig. 1).

Reasoning that regions of the protein conserved between enterobacteria, plant chloroplast, and eucaryotic RNA polymerase would probably be conserved in the RNA polymerase subunit homologs of other gram-negative organisms, we synthesized degenerate oligonucleotides from these regions to use as primers (Fig. 1 and Materials and Methods) in a PCR reaction to amplify the corresponding region from chlamydiae. With the  $\beta$  subunit primers, a discrete PCR product was generated in a reaction in which the MoPn strain of chlamydial DNA was the template and was identical in size to the corresponding fragment generated by a PCR in which *E. coli* DNA was the template (data not shown); however, no reaction products were seen when the  $\alpha$  or  $\beta'$  primer was used in a PCR with MoPn DNA as the template (data not shown).

The PCR product directed by the  $\beta$  primers was 5'-end labeled with T4 polynucleotide kinase in the presence of [ $\gamma$ - $^{32}$ P]ATP and was used as a probe to screen a plasmid library of chlamydial EcoRI fragments of MoPn DNA cloned into the vector pUC8. Ten positive clones were found after screening 900 colonies; restriction digest analysis demonstrated that all 10 clones contained the same 6.5-kilobase (kb) EcoRI fragment insert. The PCR fragment was cloned into pGEM7zf (see Materials and Methods), radiolabeled by nick translation, and used to probe Southern blots of MoPn genomic DNA cleaved with EcoRI, BamHI, or HindIII. A single band of hybridization was observed to chlamydial DNA cut with each of these enzymes, suggesting that the sequences detected are present in a single copy of the chlamydial genome (Fig. 2, lanes 1 to 3). The cloned 6.5-kb EcoRI fragment hybridized to the PCR-generated probe (lane 6) and comigrated with the genomic EcoRI fragment (lane 1) that hybridized to the PCR probe. The PCR-generated  $\beta$  probe did not cross-hybridize to *E. coli* DNA (lane 4) or human DNA (lane 5) under stringent hybridization and wash conditions. The latter control was carried out to verify that the cloned PCR product was indeed of chlamydial origin, as all preparations of chlamydial DNA are contaminated with host cell (in this case, HeLa) DNA.

Evidence that the 6.5-kb EcoRI fragment encodes the chlamydial  $\beta$  subunit gene. Limited DNA se-

<i>E. coli</i> $\alpha$ subunit	67 HEYST	110 VTAADI	
<i>B. subtilis</i> $\alpha$ subunit	63 HEFST	107 VTAADI	
Liverwort $\alpha$ subunit	72 HEYST	116 iTAcDI	
Tobacco $\alpha$ subunit	72 HEYST	116 VTAqDI	
Vaccinia virus	469 fEYra	513 qkmfsn	
PCR PROBE	HEYST 5'	<u>VTA</u> A/ODI 3'	
<i>E. coli</i> $\beta$ subunit	684 NMQRQ	809 GYNFED	
Liverwort $\beta$ subunit	542 NMQRQ	657 GYNFED	
Tobacco $\beta$ subunit	547 NMQRQ	662 GYNsED	
PCR PROBE	<u>NMORQ</u> 5'	<u>GYN</u> FED 3'	
<i>E. coli</i> $\beta'$ subunit	345 GKRVD	433 GIQAF	458 NADFD
Liverwort $\beta'$ subunit	373 GKRVD	452 GIQAF	487 NADFD
<i>D. melanogaster</i> PolII	241 GKRVD		
<i>S. cerevisiae</i> PolII			480 NADFD
Vaccinia Pol			413 NADFD
PCR PROBE	<u>GKRVD</u> 5'	<u>GIQAF</u> 5'/3'	<u>NADFD</u> 3'

FIG. 1. Conserved regions in the core polymerase  $\alpha$ ,  $\beta$ , and  $\beta'$  subunit proteins of *E. coli*. The  $\alpha$  subunit of *E. coli* (21) is compared with the *B. subtilis*  $\alpha$  subunit (5), the liverwort (*Marchantia polymorpha*) chloroplast  $\alpha$  subunit (18), the common tobacco plant chloroplast  $\alpha$  subunit (28), and the vaccinia virus RNA polymerase (7). The  $\beta$  subunit of *E. coli* (6, 19) is compared with the liverwort chloroplast  $\beta$  subunit (18) and the common tobacco plant chloroplast  $\beta$  subunit (17). The  $\beta'$  subunit of *E. coli* (20) is compared with the liverwort chloroplast  $\beta'$  subunit (18), RNA polymerase II (PolII) from *D. melanogaster* (4) and *S. cerevisiae* (1), and the vaccinia virus RNA polymerase (Pol) (7). Amino acids conserved among all or most of the species compared are denoted by capital letters, and the PCR probes used in the studies are underlined. The degenerate oligonucleotide primer derived from the peptide sequence GIQAF was used as both a 5' and a 3' primer in PCRs attempting to amplify the chlamydial  $\beta'$  gene. The numbers refer to the amino acid position in the protein.

quencing was carried out on portions of this 6.5-kb EcoRI fragment to verify that it encoded the  $\beta$  subunit gene homolog of *C. trachomatis*. Figure 3A illustrates the sequencing strategy and demonstrates that regions of this chlamydial DNA fragment encoded a protein with homology to the *E. coli*  $\beta$  subunit of RNA polymerase (amino acids 644

to 812, Fig. 3B). There was also significant homology to the plant chloroplast RNA polymerase  $\beta$  subunit (data not shown). Notably, the amino acid sequence predicted from the DNA sequence upstream of the region from which the 5' primer was chosen also conserved the homology observed between the *E. coli* and plant chloroplast  $\beta$  subunit proteins.

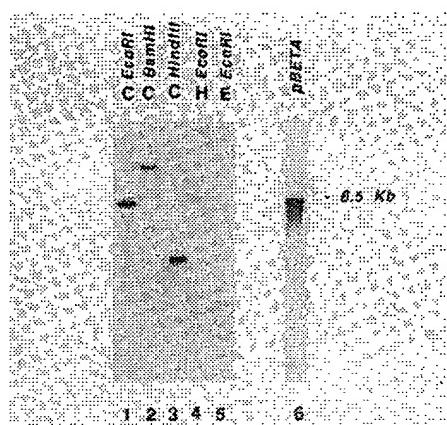


FIG. 2. Southern blot analysis of chlamydial DNA and the cloned putative  $\beta$  subunit fragment probed with the PCR products. MoPn DNA (1  $\mu$ g) (lanes 1 to 3), 5  $\mu$ g of HeLa cell DNA (lane 4), 1  $\mu$ g of *E. coli* DNA (lane 5), or 100 ng of pBETA (lane 6) was cleaved by EcoRI (lanes 1, 4, 5, and 6), BamHI, or HindIII, electrophoresed on a 1% agarose gel, and transferred to a nylon filter. Lanes 1 to 5 were hybridized to  $10^6$  cpm of a  $^{32}$ P-labeled DNA probe made by nick translation of pBETA. Lane 6 was hybridized to  $10^6$  cpm of a probe made by 5'-end labeling of the PCR products by T4 polynucleotide kinase in the presence of [ $\gamma$ - $^{32}$ P]ATP. Southern blot analysis was carried out as described previously (9).

Sequencing in from the *Cla*I site (Fig. 3A) revealed an additional region of amino acid homology to the *E. coli* and plant chloroplast proteins (amino acids 442 to 568, Fig. 3B). Together, these results leave little doubt that the cloned sequences indeed represent the chlamydial  $\beta$  subunit.

Sequencing around other restriction enzyme sites in the 6.5-kb EcoRI fragment generated two additional observations. First, upstream of the  $\beta$  subunit gene were sequences coding for a protein homologous to the eubacterial ribosomal L7/L12 protein (Fig. 3C). The *C. trachomatis* homolog was 41% identical to the *E. coli* protein; an additional 43% of the residues were conserved. Interestingly, the chlamydial L7/L12 protein was even more closely related to the cognate protein in the bacterium *Desulfovibrio vulgaris*. These two proteins were 51% identical over the 122 amino acid residues (data not shown). Second, downstream of the  $\beta$  subunit gene was found the gene encoding the  $\beta'$  subunit homolog. The amino acid sequence surrounding the *Xba*I site showed 48% identity over a 116-amino-acid stretch with the *E. coli*  $\beta'$  subunit, strongly suggesting that this gene does encode the  $\beta'$  subunit of *C. trachomatis* RNA polymerase (Fig. 3D). This gene order, L7/L12- $\beta$ - $\beta'$ , is reminiscent of the *rpoBC* operon of *E. coli* (21), which sequentially encodes L11, L1, L10, L7/L12,  $\beta$ , and  $\beta'$  in the 5' to 3' direction. Though we have not determined whether L11, L1, and L10 are encoded on DNA that is adjacent to the 5' end of the chlamydial 6.5-kb EcoRI fragment, our findings indicate that part, if not all, of the structure of this operon is conserved in chlamydiae.

**$\beta$  subunit gene of chlamydial RNA polymerase encodes a 150-kDa protein.** The 6.5-kb EcoRI fragment and the 5' EcoRI-*Nsi*I and 5' EcoRI-*Cla*I fragments derived from this EcoRI fragment were subcloned into the vector pGEM7zf so that the coding regions of these genes lay downstream of the bacteriophage T7 promoter (plasmids pBETA, p280, and p291, respectively; Fig. 4C). These plasmids were transformed into a strain of *E. coli* containing a pGEM-compatible plasmid, pGP1-2, that expresses the T7 polymerase gene

under control of a lambda promoter and a thermostable lambda repressor (cI857) (32). Upon thermoinduction of strain HMS262(pGP1-2, pBETA), pBETA directed the synthesis of an approximately 150-kilodalton (kDa) (Fig. 4A, lane 3) not seen in the control strain containing pGEM7zf [HMS262(pGP1-2, pGEM7zf)] without an insert (lane 6). This presumptive  $\beta$  protein comigrated with the lower band of a protein doublet seen in [ $^{35}$ S]methionine-pulse-labeled chlamydia-infected cells (lane 2); this doublet had the mobility characteristic of  $\beta$  (lower band) and  $\beta'$  (upper band) proteins observed in eubacteria. In some experiments (though not in the gel chosen for Fig. 4), HMS262(pGP1-2, pBETA) was observed to direct the synthesis of an additional protein product of approximately 40 kDa which most likely represents the truncated  $\beta'$  protein product that pBETA is predicted to encode. Strains bearing plasmids p280 and p291, which contain C-terminal deletions of the  $\beta$  gene, directed the synthesis of protein products of appropriately sized truncated polypeptides (ca. 80 and 42 kDa, respectively; lanes 4 and 5). The 40-kDa protein product encoded by HMS262(pGP1-2, pBETA) was not present in the radiolabeled protein products synthesized by strains bearing p280 and p291 (data not shown), corroborating the identification of the 40-kDa protein as the truncation product directed by the N-terminal fragment of the  $\beta'$  gene present in pBETA.

**Generation and characterization of a rabbit polyclonal antiserum raised to the chlamydial  $\beta$  subunit protein.** Strains HMS262(pGP1-2, p280) and especially HMS262(pGP1-2, p291) reproducibly yielded larger amounts of the newly synthesized  $\beta$  subunit protein fragment than did HMS262 (pGP1-2, pBETA) (which encodes the full-length  $\beta$  polypeptide). We chose to purify the polypeptide encoded by p291 in strain HMS262(pGP1-2) for injection into rabbits. Large quantities of the strain HMS262(pGP1-2, p291) were thermoinduced for expression, and the detergent-insoluble proteins from these cells were electrophoresed on SDS-PAGE gels (see Materials and Methods). The band corresponding to the thermoinduced  $\beta$  polypeptide fragment was excised from the gel and eluted for injection into rabbits. A polyclonal antiserum was obtained after several boostings that specifically recognized the appropriately sized  $\beta$  subunit fragments on immunoblots of *E. coli* extracts from strain HMS262 carrying pGP1-2 plus the p280 or p291 plasmid (Fig. 4B, lanes 2 and 3). On this immunoblot, the antiserum did not recognize the full-length chlamydial  $\beta$  protein synthesized by pBETA; the likeliest explanation for this observation is that synthesis of the full-length  $\beta$  protein by pBETA is very inefficient compared with that of the truncated products produced by p291 and p280 and may have been below the detection limit of this immunoblot.

**Immunoprecipitation of RNA polymerase from chlamydia-infected cells by the  $\beta$  antibody.** The antibody to the  $\beta$  subunit was produced to assist in the purification of RNA polymerase from chlamydiae; we therefore asked whether the  $\beta$  antibody could selectively immunoprecipitate the entire multisubunit enzyme. Cell lysis conditions were selected to minimize the amount of detergent in an effort to prevent dissociation or denaturation of the multisubunit enzyme (10% glycerol and 0.2% Triton X-100; see Materials and Methods). Figure 5 illustrates an immunoprecipitation of extracts prepared from cells infected with chlamydiae for 8 to 20 h, labeled with [ $^{35}$ S]methionine, and electrophoresed on 10% SDS-PAGE gels. Lanes 15 and 18 illustrate that immune serum selectively immunoprecipitated a 150-kDa protein that comigrated with the lower band of the charac-

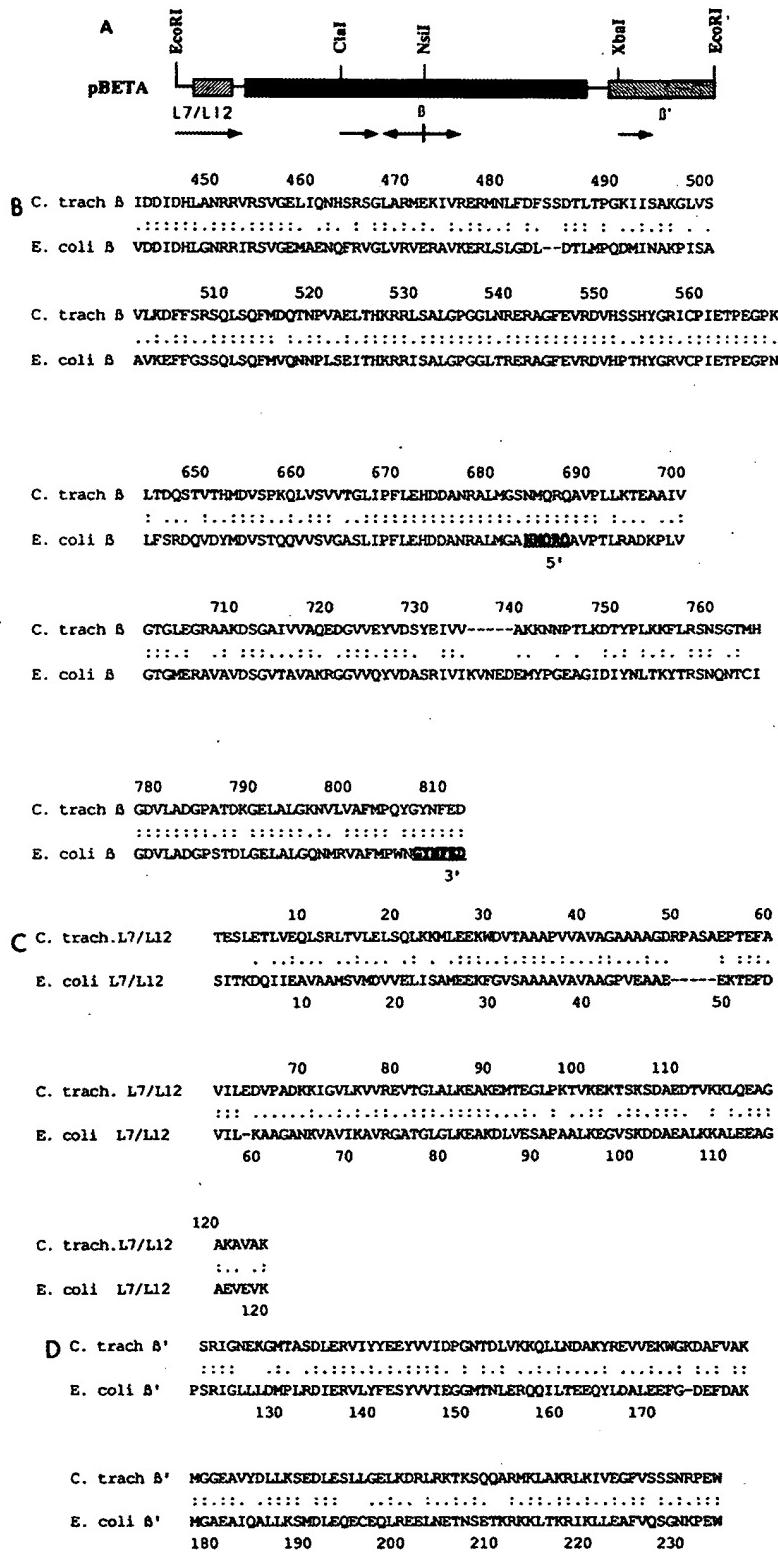
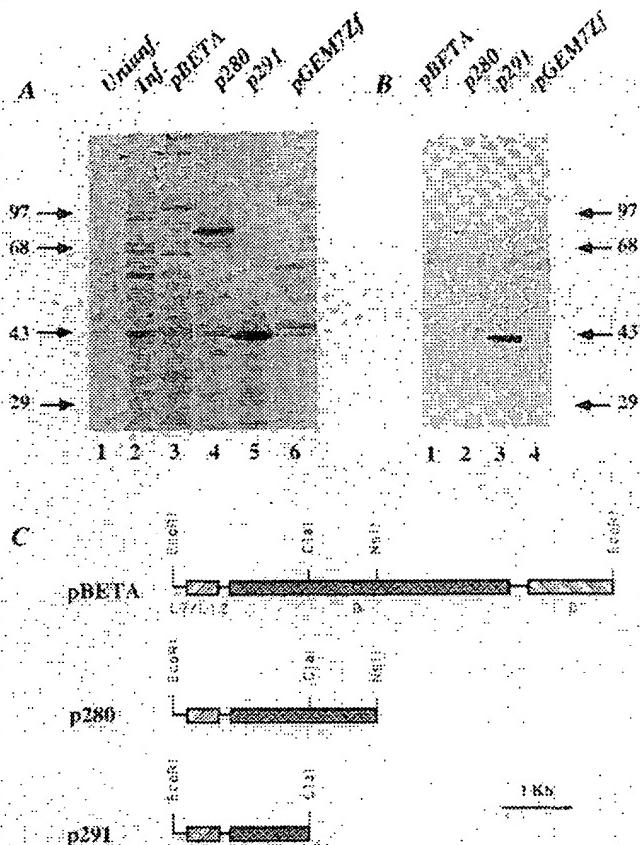
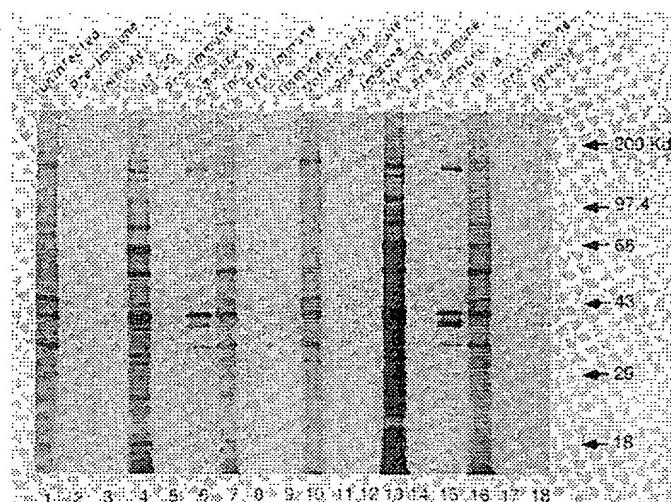


FIG. 3. (A) Organization of the pBETA clone and sequencing strategy. Arrows indicate the regions of pBETA whose sequence was determined by the chain termination method (23). Selected regions of the predicted amino acid sequence of the *C. trachomatis* (C. trach.)  $\beta$  (B), L7/L12 (C), and  $\beta'$  (D) proteins are shown and are compared with the corresponding region of the cognate *E. coli* protein. Identical amino acids are designated by two dots, and conserved amino acids are indicated by a single dot. The portion of the  $\beta$  sequence from which the  $\beta$  PCR primers were derived is underlined and highlighted. Note that the derived amino acid sequence for the *C. trachomatis*  $\beta$  protein, shown in the upper portion of panel B, corresponding to the *E. coli*  $\beta$  subunit residues 644 to 812, is missing the amino acid residues between 766 and 777.



**FIG. 4.** Overexpression of the *C. trachomatis*  $\beta$  protein in *E. coli*. (A) Autoradiograph of the SDS-PAGE gel of plasmid-encoded gene products that were thermoinduced for expression in a strain of *E. coli* containing the T7 polymerase gene on a plasmid (pGP1-2) (32) plus pBETA (lane 3), p280 (lane 4), p291 (lane 5), or pGEM7zf (lane 6), as described in Materials and Methods. Lanes 1 and 2 show the protein products labeled with [ $^{35}$ S]methionine (500  $\mu$ Ci/ml) in the presence of cycloheximide (50  $\mu$ g/ml) from uninfected HeLa cells (lane 1) or chlamydia-infected HeLa cells at 18 h.p.i. (lane 2). The full-length  $\beta$  polypeptide is indicated by the arrow in lanes 2 and 3. Although on this autoradiograph the  $\beta$  polypeptide expressed in strain HMS262(pGP1-2, pBETA) appears to comigrate with the upper band of the doublet, on SDS-PAGE gels that better resolved the doublet, it clearly migrated with the lower band. The truncated  $\beta$  polypeptides encoded by plasmids p280 and p291 are indicated by a dot. Sizes are shown in kilodaltons. (B) Western blot of a gel similar to that shown in panel A and immunoblotted to the  $\beta$  antisera. The  $\beta$  antisera was incubated with *E. coli* lysate (200  $\mu$ g/ml) for 30 min prior to binding to the immunoblot. Plasmids: pBETA (lane 1), p280 (lane 2), p291 (lane 3), and pGEM7zf (lane 4). The truncated protein products specifically recognized by the antisera are shown by a dot. (C) Structure and relevant restriction sites in the clones used for expression of the  $\beta$  subunit in *E. coli*. The shaded regions represent the coding regions of the L7/L12,  $\beta$ , and  $\beta'$  genes, as marked. The construction of the clones is described in Materials and Methods.

teristic 150-kDa doublet (lane 13); preimmune serum did not immunoprecipitate the 150-kDa polypeptide (lane 14). Likewise, no radiolabeled protein products from uninfected HeLa cells were immunoprecipitated by the immune or preimmune serum (lanes 11 and 12). Several other bands were visible in the immunoprecipitate of chlamydia-infected cells (lanes 15 and 18); they may represent some of the other subunits of RNA polymerase ( $\alpha$  or  $\sigma$ ), degradation products



**FIG. 5.** Immunoprecipitation of chlamydia-infected HeLa cell extracts with the  $\beta$  antibody in the presence or absence of cross-linking. In lanes 1 to 9, the cells were briefly exposed to the reversible chemical cross-linker DPBT, as described in Materials and Methods. Lane 1, Lysate from [ $^{35}$ S]methionine-labeled uninfected HeLa cells. Lane 2, Immunoprecipitation of [ $^{35}$ S]methionine-labeled uninfected HeLa cells with preimmune serum. Lane 3, Immunoprecipitation of [ $^{35}$ S]methionine-labeled uninfected HeLa cells with immune serum. Lane 4, Lysate from infected HeLa cells pulse-labeled from 12 to 20 h.p.i. Lane 5, Immunoprecipitation with preimmune serum of infected HeLa cells pulse-labeled from 12 to 20 h.p.i. Lane 6, Immunoprecipitation with immune serum of infected HeLa cells pulse-labeled from 12 to 20 h.p.i. Lane 7, Lysate from infected HeLa cells pulse-labeled from 4 to 8 h.p.i. Lane 8, Immunoprecipitation with preimmune serum of HeLa cells pulse-labeled from 4 to 8 h.p.i. Lane 9, Immunoprecipitation with immune serum of HeLa cells pulse-labeled from 4 to 8 h.p.i. Lanes 10 to 18, No cross-linking prior to immunoprecipitation. Lane 10, Lysate from [ $^{35}$ S]methionine-labeled uninfected HeLa cells. Lane 11, Immunoprecipitation of [ $^{35}$ S]methionine-labeled uninfected HeLa cells with preimmune serum. Lane 12, Immunoprecipitation of [ $^{35}$ S]methionine-labeled uninfected HeLa cells with immune serum. Lane 13, Lysate from infected HeLa cells pulse-labeled from 12 to 20 h.p.i. Lane 14, Immunoprecipitation with preimmune serum of infected HeLa cells pulse-labeled from 12 to 20 h.p.i. Lane 15, Immunoprecipitation with immune serum of infected HeLa cells pulse-labeled from 12 to 20 h.p.i. Lane 16, Lysate from infected HeLa cells pulse-labeled from 4 to 8 h.p.i. Lane 17, Immunoprecipitation with preimmune serum of HeLa cells pulse-labeled from 4 to 8 h.p.i. Lane 18, Immunoprecipitation with immune serum of HeLa cells pulse-labeled from 4 to 8 h.p.i. Following immunoprecipitation, the specifically bound proteins were eluted from the protein A-Sepharose by boiling in 40  $\mu$ l of 2 $\times$  Laemmli buffer (12). The entire sample was loaded on a 10% SDS-polyacrylamide gel and fluorographed. Lanes 1, 4, and 7 represent 1/50 of the cell lysate precipitated in the remaining lanes. Protein molecular mass standards are indicated on the right.

of the  $\beta$  subunit, or cross-reactivity with other chlamydial proteins. The first possibility is unlikely, as this immun serum did not reproducibly precipitate the  $\beta'$  subunit of RNA polymerase. The other two hypotheses have not been evaluated further.

We next asked whether the polymerase holoenzyme could be immunoprecipitated by the  $\beta$  antisera if the infected cells were exposed first to a cross-linking agent. While this approach would not be useful for the purification of chlamydial RNA polymerase, it could potentially identify the  $\alpha$  and  $\sigma$  subunits of this enzyme. Chlamydia-infected HeLa cells were labeled with [ $^{35}$ S]methionine and then briefly

exposed to the cleavable cross-linker DTBP. Lysates of cells infected for 8 or 20 h were immunoprecipitated with preimmune or  $\beta$  antisera and compared with lysates of cells that had undergone the same treatment except that the cross-linking step had been omitted. An additional labeled protein band was detectable in the immunoprecipitates of cross-linked lysates from chlamydia-infected cells (Fig. 5, lanes 9 and 6) that was absent from uninfected cells (Fig. 5, lane 3). This band comigrated with the upper band of the characteristic  $\beta$ - $\beta'$  doublet and presumably represents the  $\beta'$  subunit of chlamydial RNA polymerase. No new labeled protein bands of the size expected for  $\sigma$  (68 kDa [8]) or  $\alpha$  (ca. 40 kDa by analogy to other prokaryotes) could be detected specifically in any of the immunoprecipitates exposed to the cross-linker DTBP (data not shown); thus, except for the presence of  $\beta'$ , there were no other differences in the [ $^{35}$ S]methionine-labeled proteins immunoprecipitated in the cross-linked compared with the non-cross-linked samples. This analysis was complicated by the fact that several minor protein species other than the  $\beta$  polypeptide were present in the immunoprecipitates that had not been cross-linked (as noted above); these bands may have obscured the visualization of a  $\sigma$  or an  $\alpha$  subunit. Additional evidence that a  $\sigma$  factor was not immunoprecipitated by the  $\beta$  antibody in bacteria exposed to the cross-linking agent derives from the observation that no new proteins were immunoprecipitated from lysates of chlamydia-infected cells subjected to a heat shock stress (data not shown).

Immunoblots demonstrate that the  $\beta$  subunit protein is detectable early. Figure 6A shows a fluorograph of an SDS-PAGE gel of HeLa cells infected with chlamydiae for various times and pulse-labeled with [ $^{35}$ S]methionine in the presence of cycloheximide. Detectable chlamydial protein synthesis was observed at 7 h postinfection (h.p.i.) and continued throughout the life cycle; this finding is consistent with previous studies (25). A doublet of polypeptides of approximately 150 kDa was present at 7 h.p.i.; this doublet had the mobility characteristic of  $\beta$  and  $\beta'$  subunits of prokaryotic RNA polymerase. Figure 6B shows an immunoblot of a similar gel; the  $\beta$  polyclonal antiserum recognized a 150-kDa protein in chlamydia-infected HeLa cells as early as 7 h.p.i.; this 150-kDa species was not recognized by preimmune serum (data not shown). A slightly faster migrating band was seen in uninfected host cells with both preimmune (data not shown) and immune sera. An additional faint band was recognized by the immune antiserum specifically in chlamydia-infected cells; this polypeptide may represent a degradation product of the  $\beta$  subunit protein.

## DISCUSSION

Characterization of the chlamydial transcriptional apparatus is of interest because prior studies have suggested that chlamydial promoter sequences differ from those previously characterized in other bacteria (9, 24, 29; Engel and Ganem, in press). In this article we describe the cloning and analysis of the  $\beta$  subunit of RNA polymerase from the murine strain of *C. trachomatis*. Using a PCR-based approach, we designed synthetic oligonucleotides to regions of this protein conserved between *E. coli* and plant chloroplast RNA polymerase and used these as primers to amplify the intervening chlamydial sequence. This PCR-generated fragment was then used as a probe to isolate a genomic fragment encoding the  $\beta$  and  $\beta'$  subunits of RNA polymerase resembles that of *E.*

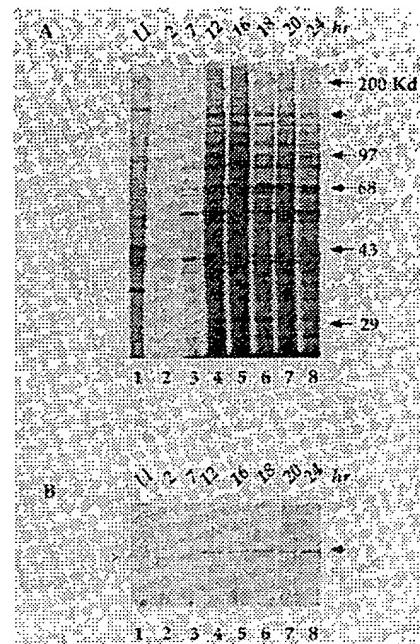


FIG. 6. Developmental immunoblot with the  $\beta$  antibody. Dishes of confluent HeLa cells were infected with chlamydiae for the indicated times and then pulse-labeled with [ $^{35}$ S]methionine for 30 min. The cells were lysed in lysis buffer (see Materials and Methods), electrophoresed on a 12% SDS-polyacrylamide gel, and transferred to nitrocellulose filters. (A) Autoradiograph of the filter. Protein sizes (in kilodaltons) are indicated to the right. The shaded arrow indicates the  $\beta$  subunit. (B) Immunoblot of the same filter with the  $\beta$  antiserum. The shaded arrow indicates the  $\beta$  subunit. The  $>$  indicates a HeLa cell protein that cross-reacts with the  $\beta$  antiserum. The  $<$  indicates a possible degradation product of the  $\beta$  subunit. Lane 1, Uninfected HeLa cells. Lanes 2 to 8, HeLa cells infected with chlamydiae for 2, 7, 12, 16, 18, 20, or 24 h, respectively.

*coli*. A similar strategy was used to isolate the chlamydial  $\alpha$  and  $\beta'$  subunit genes by PCR but was unsuccessful. Perhaps the regions from which the degenerate oligonucleotide primers were derived are not conserved in the chlamydial homologs. It is also formally possible that chlamydiae entirely lack an  $\alpha$  homolog. Alternatively, some feature of the oligonucleotides (such as secondary structure) may have prevented them from serving as effective primers in the PCR (J. Engel, unpublished observations).

The chlamydial  $\beta$  subunit gene directed the synthesis of a 150-kDa protein product when expressed in a strain of *E. coli* that depended upon thermoinduction for expression of plasmid-encoded genes. Interestingly, pBETA and its derivatives could not be transformed into a strain of *E. coli* (31), where induction, though still dependent on T7 polymerase for expression, is under the control of a *lac* promoter (F. Malik and J. Engel, unpublished observations). The chlamydial  $\beta$  and  $\beta'$  products were not toxic to *E. coli* in the HMS262(pGP1-2) background, as strains containing these constructs could be grown stably at 42°C (F. Malik and J. Engel, unpublished observations). Attempts by others to express in *E. coli* a mutant  $\beta$  subunit of *E. coli* RNA polymerase were successful only when induction was dependent upon heat shock. These workers hypothesized that the high temperature of heat shock denatured the mutant polypeptide, rendering it insoluble and thus unavailable to compete with the wild-type subunit for assembly into the core enzyme (13). Similar explanations may account for our

ability to overproduce the chlamydial  $\beta$  subunit only in thermoinducible strains. It is interesting that pBETA could be cloned in TG1 (Gibson, Ph.D. thesis), an *E. coli* strain lacking the T7 RNA polymerase gene; we suspect that there is very little  $\beta$  subunit expression in this background, where the only route for chlamydial gene expression is by RNA polymerase initiating transcription from a plasmid promoter.

Attempts to characterize the expression of  $\beta$  subunit RNA by Northern (RNA) blot analysis and S1 nuclease analysis have been unsuccessful (J. Engel, unpublished observations). This finding suggests that the mRNA is either of very low abundance or very unstable. Similar results were obtained during studies of the expression of the cloned major vegetative  $\sigma$  factor from *C. trachomatis* (8). A truncated  $\beta$  polypeptide was purified from a strain of *E. coli* geared for overexpression of plasmid-encoded proteins and used to raise a polyclonal antiserum in rabbits. Probing of developmental immunoblots with this antiserum demonstrated that the  $\beta$  subunit protein was detectable as early as 7 h.p.i., the earliest time at which protein synthesis has been reproducibly detected *in vivo* in chlamydiae. Although we could not detect the  $\beta$  subunit at 2 h.p.i. on these immunoblots, we presume that EBs harbor at least a few molecules of RNA polymerase, perhaps synthesized during the previous cycle of replication. These polymerase molecules, then, would initiate the transcription of the earliest genes during the next round of intracellular chlamydial replication.

The characterization of the transcriptional machinery from other eubacteria has depended on direct biochemical purification of bacterial polymerase combined with assaying the holoenzyme by *in vitro* transcription on specific templates. This approach is not feasible for chlamydiae, as the organism grows so poorly in culture that it would be exceedingly difficult to generate the necessary starting material for such large-scale enzyme purifications. We initiated this study with the presumption that this  $\beta$ -specific antiserum would be of assistance during the biochemical purification of chlamydial RNA polymerase, for example, for use during an affinity purification procedure. This strategy has been used successfully for the purification of other bacterial RNA polymerases (22, 30). Our results, however, demonstrate that this particular antibody is able to immunoprecipitate only the  $\beta$  subunit of RNA polymerase. Several explanations are possible for this observation. The enzyme may dissociate during the immunoprecipitation under our lysis and wash conditions. Notably, though, washing the immunoprecipitates under gentler conditions (150 mM NaCl) did not allow the immunoprecipitation of intact core enzyme (J. Engel, unpublished observations). Alternatively, this antiserum may only recognize epitopes on unassembled  $\beta$  polypeptide chains or epitopes that are masked when the core or holoenzyme assembles. Relevant to this last point is the fact that the antiserum was raised to the N-terminal portion of the  $\beta$  subunit.

We also asked whether the other subunits of RNA polymerase could be immunoprecipitated if chlamydia-infected cells are exposed to a cross-linking agent prior to immunoprecipitation. While such an approach would not be directly applicable to the purification of functional holoenzyme, it could provide a convenient way to identify alternative  $\sigma$  factors associated with RNA polymerase at various times during the life cycle or under different environmental conditions. We were unable, however, to identify the  $\sigma$  subunit in such immunoprecipitates, even under conditions in which a new  $\sigma$  factor might be expected to stably associate with core enzyme (e.g., heat shock [J. Engel, unpublished observa-

tions] or early in the intracellular developmental life cycle). As the majority of RNA polymerase molecules in a cell exist as core enzyme, we may not be able to detect a  $\sigma$  polypeptide above the background level of other nonspecifically immunoprecipitated proteins. Whether the  $\sigma$  subunit was coprecipitated could not be ascertained, because several proteins of ca. 40 kDa were precipitated specifically by the immune serum. One of these proteins is probably the major outer membrane protein, which binds nonspecifically to protein A-Sepharose (Richard Stephens, personal communication) and thus contaminates the precipitates.

Based on our success in expressing the chlamydial  $\beta$  and  $\sigma$  proteins in *E. coli* (8), we are now directing our efforts to cloning and expressing the  $\alpha$  and  $\beta'$  subunits in *E. coli* with the ultimate goal of coexpression of all the chlamydial RNA polymerase subunits in *E. coli*. This approach will allow the reconstitution of chlamydial RNA polymerase in *E. coli* for use in *in vivo* transcription or *in vitro* transcription assays on authentic chlamydial templates, enabling us to further study the *cis* elements and *trans*-acting factors that underlie the genetic regulation of the chlamydial life cycle.

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